CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

SAFE DRINKING WATER AND TOXIC ENFORCEMENT ACT OF 1986
(PROPOSITION 65)

NOTICE TO INTERESTED PARTIES
February 19, 1999

Availability of Draft Data Summaries and Draft Priorities for Chemicals With Respect to Their Potential to Cause Cancer: Request for Relevant Information

The California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA), as lead agency for the implementation of the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65), has developed a procedure for prioritizing chemicals for consideration under Proposition 65 by the "State's qualified experts". Two committees of the Science Advisory Board (SAB), known as the Carcinogen Identification Committee, and the Developmental and Reproductive Toxicant (DART) Identification Committee, serve as the State's qualified experts for rendering an opinion as to whether a chemical is known to the State to cause cancer, birth defects or other reproductive harm.

The procedure used by OEHHA to identify, prioritize and select candidate chemicals for evaluation by the SAB Committees is described in, "Procedure for Prioritizing Candidate Chemicals for Consideration Under Proposition 65 by the State's Qualified Experts," May 1997 and is available on the Internet at http://www.oehha.ca.gov. In accordance with this procedure, prioritized chemicals with a final priority of High Carcinogenicity Concern are assigned to the Candidate List, from which chemicals will be chosen for the preparation of hazard identification documents, and subsequent evaluation by the Carcinogen Identification Committee. All chemicals not assigned a final "high" level of carcinogenicity concern are assigned to Category II. In a slight change from previous releases, where draft priorities of Medium High, Medium, and Low Carcinogenicity Concern were stated explicitly in the data summaries, here draft priorities are identified as either "High" Carcinogenicity Concern, or "Not High" enough to merit placement on the Candidate List. We are implementing this change following the recognition that considerable resources have been expended by both the interested public and the State in the course of providing and responding to comments not pertinent to the placement of chemicals on or off the Candidate List. The main purpose of this phase of the prioritization process is to decide which chemicals should appear on the Candidate List; many of the comments received on previous releases focused on refining the priority of chemicals within Category II. We welcome comments from the public on this change.

With this notice we are announcing the release of draft data summaries and draft priorities concerning the potential for the chemicals indicated below to cause cancer. This notice initiates a 60-day public comment period. These 60 chemicals were selected for prioritization as described in OEHHA's Notice to Interested Parties: Results of the Second Round Pilot Random Selection of the Chemicals Eligible for Prioritization For Consideration of Carcinogenicity Evaluation, published in

the *California Regulatory Notice Register* on April 3, 1998. The chemicals and their draft priorities are as follows:

Name of Chemical	CAS No.
On Candidate List due to HIGH CARCINOGENICITY CONCERN	
allyl isovalerate	2835-39-4
4-*bis*(2-hydroxyethyl)amino-2-(5-nitro-2-thienyl)-quinazoline	33372-39-3
bleomycin	11056-06-7
1-butylhydrazine hydrochloride	56795-65-4
carboxymethylnitrosourea	60391-92-6
3-chloromethylpyridine hydrochloride	6959-48-4
chrysoidine	532-82-1
N,*N'*-diethylthiourea	105-55-5
3,3'-dimethoxybenzidine-4,4'-diisocyanate	91-93-0
dimethyldiazene-1-oxide (methylazoxymethane / azoxymethane)	25843-45-2
estradiol mustard	22966-79-6
N'-ethyl-*N*-methyl-*N*-nitrosourea	72479-13-1
N'-ethyl-*N*-nitrosobutylamine	4549-44-4
4-ethylsulfonylnaphthalene-1-sulfonamide	842-00-2
hexachlorobutadiene	87-68-3
ICRF-159	21416-87-5
isophosphamide	3778-73-2
lovastatin	75330-75-5
N-(2-methoxyethyl)-*N*-nitrosourea	108278-70-2
3'-methyl-4-dimethylaminoazobenzene	55-80-1
methylphenidate and its hydrochloride (Ritalin)	113-45-1
4-methylquinoline	491-35-0
MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone)	77439-76-0
6-nitrobenzimidazole	94-52-0
N-nitrosomethyl-*N*-heptylamine	16338-99-1
N-nitroso-*N*-pentylurea (*N*-amyl-*N*-nitrosurea)	10589-74-9
petasitenine	60102-37-6
phenelzine and its acid salts	156-51-4
pivalolactone	1955-45-9
pyrrolizidine alkaloids which are metabolized to dehydroretronecine or dehydroheliotridine	---
sesamol	533-31-3
styrene	100-42-5
tetrachlorvinphos	22248-79-9
2,4,6-trimethylaniline and its hydrochloride (aminomesitylene)	88-05-1

Name of Chemical	CAS No.
Category II (Not HIGH CARCINOGENICITY CONCERN)	
2-amino-5-nitrothiazole	121-66-4
11-aminoundecanoic acid	2432-99-7
antipyrine (phenazone)	60-80-0
p-benzoquinone dioxime	105-11-3
C.I. acid blue 9 and its salts	2650-18-2
C.I. acid red 51	16423-68-0
chlorinated paraffins (C_{23}; 43% chlorine)	108171-27-3
4-chloro-4'-aminodiphenyl ether	101-79-1
4-chloro-*m*-phenlyenediamine	5131-60-2
dibromomannitol	488-41-5
diclofop-methyl	51338-27-3
diltiazem	42399-41-7
FD&C blue no. 2	860-22-0
malathion	121-75-5
6-methoxy-2-nitronaphtho[1,8-bc]pyran	10502-39-9
mexacarbate	315-18-4
omeprazole	73590-58-6
tocopherol mix (E-mix 80)	1406-66-2
triadimenol	55219-65-3
tribenuron methyl	101200-48-0
trimethylthiourea	2489-77-2
tris(2-ethylhexyl)phosphate (trioctyl phosphate)	78-42-2
troysan polyphase (IPBC)	55406-53-6
INADEQUATE DATA to establish level of concern	
1-butanol (*n*-butanol, *n*-butyl alcohol)	71-36-3
2-bromo-2-methylpropane (*tert*-butyl bromide)	507-19-7
POSTPONED	
bis(4-chlorophenyl)sulfone (*p,p'*-dichlorophenylsulfone)	80-07-9

OEHHA also announces that a public workshop to receive external scientific peer review and public comments on the draft data summaries and draft priority assignments for these chemicals will be held on **Friday, April 9, 1999**. The workshop will commence at 10:00 a.m. in Conference Room A, Elihu Harris State Building, 1515 Clay Street, Oakland California, and will last until all business has been conducted or until 5:00 p.m.

OEHHA is committed to public participation and external scientific peer review in its implementation of Proposition 65, and welcomes public input. The draft data summaries and draft priority assignments for these chemicals are available from the Proposition 65 Implementation Office at the address and telephone number indicated below, or from the Internet at the following address: http://www.oehha.ca.gov.

Written comments may be submitted **in triplicate** to:

Cynthia Oshita
Office of Environmental Health Hazard Assessment
301 Capitol Mall, 2nd Floor
Sacramento, California 95814
FAX (916) 327-1097
(916) 445-6900

In order to be considered, comments must be postmarked (if sent by mail) or received at OEHHA (if delivered in person or sent by FAX) by 5 p.m. Tuesday, April 20, 1999.

DRAFT PRIORITIZED CANDIDATE CHEMICALS UNDER CONSIDERATION FOR CARCINOGENICITY EVALUATION:

BATCH #3

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

February 1999

Draft data summaries for 60 chemicals under consideration for carcinogenicity evaluation ("Batch #3") have been prepared and are presented here. The same process utilized to select the previous group of chemicals prioritized for carcinogenicity concern (i.e., Batch #2) was employed in the selection of the Batch #3 chemicals. Namely, 60 chemicals were randomly selected from 100 chemicals in the tracking database. The 100 chemicals were those for which toxicity information had been entered into the toxicity field of the data entry sheet. Prioritization of Batch #3 chemicals proceeded as described in the document entitled "Procedure for Prioritizing Candidate Chemicals for Consideration Under Proposition 65 by the State's Qualified Experts" (May 1997). Today marks the start of a 60-day public comment period on the draft data summaries for these 60 selected chemicals. A public workshop will be held on April 9, 1999 to receive verbal comments. Public comments received on the data summaries during the 60-day comment period will be reviewed and considered as part of the assignment of final priorities.

Prioritized chemicals with a final priority of "High" Carcinogenicity Concern are assigned to the Candidate List, from which chemicals will be chosen for the preparation of hazard identification documents. All chemicals not assigned a final "high" level of carcinogenic concern are assigned to Category II. Action is not anticipated on Category II chemicals until all high priority chemicals on the Candidate List with known or potential exposure have been evaluated. At that point, with Committee and public input, OEHHA will refine the existing process in order to determine which of the Category II prioritized chemicals should be brought forward for consideration by the Carcinogen Identification Committee.

In a slight change from previous releases, where draft priorities of Medium High, Medium, and Low Carcinogenicity Concern were stated explicitly in the data summaries, here draft priorities are identified as either "High" Carcinogenicity Concern, or "Not High" enough to merit placement on the Candidate List. We are implementing this change following the recognition that considerable resources have been expended by both the interested public and the State in the course of providing and responding to comments not pertinent to the placement of chemicals on or off the Candidate List. The main purpose of this phase of the prioritization process is to decide which chemicals should appear on the Candidate List; many of the comments received on previous releases focused on refining the priority of chemicals within Category II. We welcome comments from the public on this change.

It should be noted that (1) this prioritization process reflects a preliminary, rather than an in-depth review of carcinogenicity and exposure data, and, (2) the process is a continuous one; efforts to gather additional information on Category I and Category II chemicals are ongoing.

Name of Chemical	CAS No.	Level of Exposure Concern	Page
On Candidate List due to HIGH CARCINOGENICITY CONCERN			
allyl isovalerate	2835-39-4	high	4
carboxymethylnitrosourea	60391-92-6	high	7
hexachlorobutadiene	87-68-3	high	9
lovastatin	75330-75-5	high	12
methylphenidate and its hydrochloride (ritalin)	113-45-1	high	14
4-methylquinoline	491-35-0	high	17
MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone)	77439-76-0	high	19
phenelzine and its acid salts	156-51-4	high	21
pyrrolizidine alkaloids which are metabolized to dehydroretronecine or dehydroheliotridine	---	high	23
sesamol	533-31-3	high	31
styrene	100-42-5	high	33
tetrachlorvinphos	22248-79-9	high	41
bleomycin	11056-06-7	medium	44
chrysoidine	532-82-1	medium	46
N,N'-diethylthiourea	105-55-5	medium	48
isophosphamide	3778-73-2	medium	50
6-nitrobenzimidazole	94-52-0	medium	53
petasitenine	60102-37-6	medium	55
1-butylhydrazine hydrochloride	56795-65-4	low	57
3,3'-dimethoxybenzidine-4,4'-diisocyanate	91-93-0	low	58
estradiol mustard	22966-79-6	low	60
pivalolactone	1955-45-9	low	62
2,4,6-trimethylaniline and its hydrochloride (aminomesitylene)	88-05-1	low	64
4-*bis*(2-hydroxyethyl)amino-2-(5-nitro-2-thienyl)-quinazoline	33372-39-3	n.i.c.	67
3-chloromethylpyridine hydrochloride	6959-48-4	n.i.c.	68
dimethyldiazene-1-oxide (methylazoxymethane / azoxymethane)	25843-45-2	n.i.c.	70
N'-ethyl-*N*-methyl-*N*-nitrosourea	72479-13-1	n.i.c.	73
N'-ethyl-*N*-nitrosobutylamine	4549-44-4	n.i.c.	75
4-ethylsulfonylnaphthalene-1-sulfonamide	842-00-2	n.i.c.	77
ICRF-159	21416-87-5	n.i.c.	80
3'-methyl-4-dimethylaminoazobenzene	55-80-1	n.i.c.	83
N-nitrosomethyl-*N*-heptylamine	16338-99-1	n.i.c.	85
N-nitroso-*N*-pentylurea (*N*-amyl-*N*-nitrosurea)	10589-74-9	n.i.c.	87
N-(2-methoxyethyl)-*N*-nitrosourea	108278-70-2	inadequate data	90

Name of Chemical	CAS No.	Level of Exposure Concern	Page
Category II (Not HIGH CARCINOGENICITY CONCERN)			
C.I. acid blue 9 and its salts	2650-18-2	high	92
C.I. acid red 51	16423-68-0	high	96
chlorinated paraffins (C_{23}; 43% chlorine)	108171-27-3	high	99
diclofop-methyl	51338-27-3	high	101
diltiazem	42399-41-7	high	103
FD&C blue no. 2	860-22-0	high	105
malathion	121-75-5	high	107
omeprazole	73590-58-6	high	113
tocopherol mix (E-mix 80)	1406-66-2	high	118
triadimenol	55219-65-3	high	120
trimethylthiourea	2489-77-2	high	121
tris(2-ethylhexyl)phosphate (trioctyl phosphate)	78-42-2	high	123
2-amino-5-nitrothiazole	121-66-4	medium	125
11-aminoundecanoic acid	2432-99-7	medium	127
antipyrine (phenazone)	60-80-0	medium	129
troysan polyphase (IPBC)	55406-53-6	medium	131
p-benzoquinone dioxime	105-11-3	low	132
4-chloro-4'-aminodiphenyl ether	101-79-1	low	134
4-chloro-*m*-phenylenediamine	5131-60-2	low	136
dibromomannitol	488-41-5	low	139
mexacarbate	315-18-4	low	141
tribenuron methyl	101200-48-0	low	143
6-methoxy-2-nitronaphtho[1,8-bc]pyran	10502-39-9	n.i.c.	144
INADEQUATE DATA to establish level of concern			
1-butanol (*n*-butanol, *n*-butyl alcohol)	71-36-3	high	145
2-bromo-2-methylpropane (*tert*-butyl bromide)	507-19-7	medium	147
POSTPONED		**Status**	
bis(4-chlorophenyl)sulfone (*p,p'*-dichlorophenylsulfone)	80-07-9	Results of NTP 2-year bioassays conducted in rats and mice expected in 1-2 years	

n.i.c. = No Identified Concern

CARCINOGENICITY DATA SUMMARY: ALLYL ISOVALERATE

Allyl isovalerate (AIV; allyl-3-methylbutyrate; 2-propenyl-3-methylbutanoate; CAS No. 2835-39-4) is a synthetic fragrance and flavoring ingredient used in various foods, cosmetics and consumer household products. It is not known to occur naturally. AIV has been approved for use in food by the US FDA (21CFR172.515). US FDA estimates that 3.3 lb of AIV are used annually in the U.S. food supply and that human (per capita) consumption is 2.8 x 10^{-6} mg/kg-day (assuming 10% of the population consumed the entire amount). It was listed as Generally Recognized as Safe (GRAS) by the Flavor and Extract Manufacturers' Association (FEMA) Expert Panel 3; the Cancer Assessment Committee decision is pending.

AIV is listed on the US EPA 1993 TSCA Inventory. Commercial production in the U.S. has only been reported since 1973; since then only one U.S. company has produced it (production data not disclosed and import data not published; IARC, 1985). One estimate of the annual production of AIV in the U.S. has been 1000 lbs/year (NTP, 1983). Although it is approved for use in foods and consumer products, a survey of U.S. industries on the use of food additives in 1977 did not indicate its use in any specific food items, and a compilation of chemicals used in cosmetics did not include this chemical. Current use for these purposes is not known.

AIV has been classified by IARC (1985) as a Group 3 carcinogen (limited evidence in animals and no studies in humans, therefore nonclassifiable). Since that evaluation, additional genotoxicity data, including observation of sister-chromatid exchanges *in vitro*, have become available.

Carcinogenicity Data available:

Epidemiological studies

No studies regarding the carcinogenicity of AIV to humans have been located in the literature.

Animal bioassays

1. Mouse long-term gavage studies: NTP, 1983. B6C3F_1 mice (50/sex/dose) were administered AIV at doses of 0, 31, or 62 mg/kg_{bw} by gavage in corn oil 5 day/week for 103 weeks. Decreased survival among female mice in the low-dose group resulted from a high incidence of genital tract infection. A significant dose-response trend for malignant lymphomas was observed in female mice with a statistically significantly increased incidence in high-dose female mice compared to controls (11/50, 22%; 11/50, 22% and 18/50, 36% for control, low-, and high-doses, respectively). Lymphomas were increased, but not significantly in male mice mice (control 4/50, low dose 6/50, high dose 8/50). A significantly increased trend ($p < 0.05$) of squamous cell papillomas of the nonglandular forestomach was observed in male mice (control 0/50, low dose 1/50, high dose 3/48). NTP concluded that under the conditions of the bioassay AIV was carcinogenic for female B6C3F1 mice.

2. Rat long-term gavage studies: NTP, 1983. F344/N rats (50/sex/dose) were administered AIV at doses of 0, 31, or 62 mg/kg_{bw} by gavage in corn oil 5 day/week for 103 weeks. A significant dose-response trend of mononuclear-cell leukemia was observed in male and female rats. Compared to controls, the incidence was significantly ($p < 0.05$) increased in high-dose male rats (1/50, 4/50 and 7/50 for control, low-, and high-doses respectively). In female rats the increases in incidences (control 4/50, low dose 6/50, high dose 9/49) were not individually significant. However the dose-related trend in leukemia incidence was significant ($p < 0.05$) by life-table analysis for both sexes. Two high-dose male rats and one control and one high-dose female rat had central gliomas: no such tumors occurred in the control or low-dose animals. NTP concluded that under the conditions of the bioassay AIV was carcinogenic for male F344/N rats.

Other relevant data

Mutagenicity was not observed in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 (0, 100-10,000 µg/plate), either with or without metabolic activation provided by Aroclor 1254-induced rat or hamster liver preparations (NTP, 1983).

NTP (1998) reported that AIV tested both positive and negative in *in vitro* cytogenetic and mutagenicity assays. Positive studies include induction of sister chromatid exchanges (SCE) and chromosome aberrations in Chinese hamster ovary cells (Gulati *et al*., 1989), an unreferenced positive result in the mouse lymphoma assay, and an unreferenced non-standard protocol study for SCE. Negative studies include an unreferenced study for chromosome aberrations, sex-linked recessive lethal/reciprocal translocations in *Drosophila* (Woodruff *et al*., 1985), and *Salmonella* mutagenicity (Mortelmans *et al*., 1986).

Among the metabolites of AIV are allyl alcohol and acrolein. Allyl alcohol has not been evaluated for carcinogenicity by US EPA (1997). Acrolein has been classified by IARC (1987) as a Group 3 carcinogen (not classifiable due to inadequate evidence in humans and experimental animals) and by US EPA (1997) as a group C (possible human carcinogen). The metabolites of allyl alcohol and acrolein are epoxides; glycidol and glycidaldehyde, respectively (NTP, 1983). Glycidaldehyde has been classified as a B2 carcinogen (possible human carcinogen) by US EPA (1997) and listed as causing cancer under Proposition 65.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over allyl isovalerate based upon evidence for a carcinogenic response in both male rats and female mice. The evidence of genotoxicity, although mixed, provides some support for the level of concern. The fact that AIV is metabolized to a chemical, glycidaldehyde, listed as causing cancer under Proposition 65 supports the level of concern.

There is a **HIGH** level of **exposure concern** for allyl isovalerate since it has been designated a GRAS food additive and may be present in food and cosmetic items. Although widespread, exposure levels would be expected to be mostly low according to US FDA calculations.

References

Gulati DK, Witt K, Anderson B, Zeiger E, Shelby M (1989). Chromosome aberration and sister chromatid exchange test in Chinese hamster ovary cells in vitro. III: Results with 27 chemicals. *Environ Mol Mutagen* **13**:133-93.

International Agency for Research on Cancer (IARC, 1985). Allylisovalerate. *IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans, Allyl Compounds, Aldehydes, Epoxides and Peroxides*. Vol. 36: pp. 69-74. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC Monograph on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs, Volumes 1-42*. Supplement 7: p 56. IARC, Lyon, France.

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E (1986). Salmonella mutagenicity tests. II. Results from the testing of 270 chemicals. *Environ Mutagen* **8**(Suppl 7): 1-119.

National Toxicology Program (NTP, 1983). Carcinogenesis studies of allylisovalerate (CAS No. 2835-39-4) in F344/N rats and B6C3F_1 mice (gavage studies). TR-253. NIH Publ. No. 83-2509. USHHS/PHS/NIH.

National Toxicology Program (NTP, 1998). Allyl isovalerate [cited 5/15/98]; [2 screens]. Available from: URL: http:\\ntp-server.niehs.nih.gov\htdocs\Results_status\Resstata\10717-G.html.

US Environmental Protection Agency (US EPA, 1997). Integrated Risk Information System (IRIS). Washington DC, USA. July 1, 1993.

Woodruff RC, Mason JM, Valencia R, Zimmering S (1985). Chemical mutagenesis testing in Drosophila: V. Results of 53 coded compounds tested for the National Toxicology Program. *Environ Mutagen* **7**: 677-702.

CARCINOGENICITY DATA SUMMARY: CARBOXYMETHYLNITROSOUREA

N-Carboxymethyl-N-nitrosourea (CMNU; nitroso-hydantoic acid; N-(aminocarbonyl)-N-nitroglycine; CAS No. 60391-92-6) is a naturally occurring compound. CMNU is formed from glycocyamine, a guanido compound present in foods, especially meat products, as a result of reaction with nitrites under acidic conditions (e.g., human stomach) (Maekawa *et al*., 1983).

Carcinogenicity Data available:

Epidemiological studies

No data on the carcinogenic effects of this chemical in humans were identified.

Animal bioassays

1. Rat 74-week drinking water study: Bulay *et al*., 1979. A group of 40 eight-week-old male MRC Wistar rats was administered CMNU as a 260 mg/L solution in drinking water five days per week for 74 weeks. Groups of 22 and 24 male rats were given the buffer solution, five days per week for 52 weeks. A group of 47 male rats given tap water throughout their lifetime served as untreated controls. Among the treated rats, increases were observed in the incidence of tumors of the gastrointestinal tract [untreated control 2/47; buffer control 0/22, 2/24; treated 9/40 ($p<0.03$)] and the skin [untreated control 1/47; buffer control 0/22, 0/24; treated 7/40 ($p<0.02$)]. Gastrointestinal tract tumors included squamous cell carcinomas of the tongue, squamous cell papillomas of the forestomach, and adenocarcinomas of the small and large intestine. Skin tumors included squamous cell papillomas and carcinomas. Increases in adrenal gland tumors (pheochromocytomas and cortical adenomas) were also reported [treated 8/40 ($p<0.03$)] but the corresponding control incidences were not clearly stated. Bulay *et al*. (1979) concluded that CMNU was a weak carcinogen in MRC Wistar rats.

2. Rat 68-week drinking water study: Maekawa *et al*., 1983. Groups of 40 eleven-week-old female Donryu rats were administered CMNU daily in the drinking water at concentrations of 0,100, 200 or 400 ppm for 68 weeks. There were significant increases and a discernible dose-response relationship in intestinal tumors, almost all of which were epithelial adenomas/adenocarcinomas of the small intestine [control 0/36, low-dose 5/40, mid-dose 19/38 ($p<0.01$), high-dose 27/34 ($p<0.01$)]. There were significant increases in mammary gland tumors (almost all were fibroadenomas with a few fibromas, adenomas, and adenocarcinomas) at the low- and mid-dose level [control 9/36, low-dose 28/40 ($p<0.01$), mid-dose 30/38 ($p<0.01$), high-dose 11/34]. In addition, mucosal hyperplasia or preneoplastic changes of the small intestine were observed with a discernible dose-response relationship [control 0/36, low dose 4/40, mid dose 12/38 ($p<0.01$), high dose 24/34 ($p<0.01$)].

Other Relevant Data:

CMNU was mutagenic with or without metabolic activation in *E. coli* (Yoshikawa *et al*., 1979) and *S. typhimurium* TA 1535, TA 98, TA 100, TA 1537 (Lee *et al*., 1977; Ishidate *et al*., 1981). It was positive for chromosomal aberrations in vitro in Chinese hamster cells, with or without metabolic activation (Ishidate *et al*., 1981). CMNU is a nitrosamine and is likely to form reactive metabolites that can alkylate DNA and proteins.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** as CMNU has been shown to induce tumors of the gastrointestinal tract in both male and female rats in two separate studies and in different strains. It also induced skin tumors in male rats and mammary gland tumors in female rats. The level of concern is reinforced by close structural similarities with other nitrosamines and by the demonstrated genotoxicity and mutagenicity of the compound.

There is a **HIGH** level of **concern** over the extent of exposure for CMNU as the precursor of this compound, glycocyamine, is present in many meat products. This precursor can react with sodium nitrite, which is frequently added to food, to form CMNU in the human stomach (Yoshikawa *et al*., 1979).

References

Bulay O, Mirvish SS, Garcia H, Pelfrene AF, Gold B, Eagen M (1979). Carcinogenicity test of six nitrosamides and a nitrosocyanamide administered orally to rats. *J Natl Cancer Inst* 62(6):1523-1528.

Ishidate M, Sofuni T, Yoshikawa K (1981). Chromosomal aberration tests in vitro as a primary screening tool for environmental mutagens and/or carcinogens. *Gann Monograph on Cancer Research* 27:95-108.

Lee K, Gold B, Mirvish SS (1977). Mutagenicity of 22 nitrosamides and selected compounds for Salmonella typhimurium TA-1535. *Mutat Res* 48:131-138.

Maekawa A, Ogiu T, Matsuoka H, Onodera K, Furuta H, Tanigawa H, Odashima S (1983). Induction of tumors in the small intestine and mammary gland of female Donryu rats by continuous oral administration of N-carboxymethyl-N-nitrosourea. *J Cancer Res Clin Oncol* 106:12-16.

Yoshikawa K, Uchino H, Yamamoto M, Yamada T, Tanimura A, Kondo S (1979). Effect of N-carboxymethyl-N-nitrosourea on viability and mutagenic response of repair-deficient strains of Escherichia coli. *Gann* 70:705-708.

CARCINOGENICITY DATA SUMMARY: HEXACHLOROBUTADIENE

Hexachlorobutadiene (hexachloro-1,3-butadiene, perchlorobutadiene, HCBD; CAS No. 87-68-3) is used primarily as a chemical intermediate in the manufacture of rubber compounds (ATSDR, 1994). Lesser quantities are used as a solvent, a fluid for gyroscopes, a heat transfer liquid, hydraulic fluid, and as a chemical intermediate in the production of chlorofluorocarbons and lubricants. Internationally, the chemical has limited use as a pesticide. It is a by-product of chlorinated solvent manufacture. It was formerly used to recover chlorine-containing gas in chlorine plants (IARC, 1979). IARC (1979; 1987) classified HCBD as a Group 3 carcinogen based on limited evidence in animals and no data in humans. This classification was based on IARC's 1979 review, and did not include review of the bioassay by Chadin *et al*., 1985, or the more recent studies on genotoxicity and mechanism. US EPA has classified it as a Group C carcinogen (EPA, 1993). NIOSH identified HCBD as a potential occupational carcinogen (NIOSH, 1994).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to HCBD were found in earlier searches by IARC (1979;1987) or more recently by OEHHA.

Animal bioassays

1. Rat long-term feeding studies: Kociba *et al*., 1977. Groups of 39-40 SPF Sprague-Dawley rats/sex/group were fed diets formulated to deliver 0.2, 2 or 20 mg HCBD/kg bw/day for 22 (males) or 24 months (females). Ninety animals/sex were fed control diets. A statistically significant increase ($p < 0.05$) in kidney tumors (renal tubular adenomas and adenocarcinomas combined) was observed in high-dose males (1/90, 0/40, 0/40, and 9/39 for control, low-, mid-, and high-dose groups, respectively) and females (0/90, 0/40, 0/40, and 6/40). The incidences of renal tubular adenocarcinomas in high-dose males and females were 9/39 and 3/40, respectively. Two of these tumors metastasized to the lungs. Increased hyperplasia of the renal tubular epithelium was observed in mid-dose males and females. The authors concluded that the renal neoplasms were related to treatment with HCBD.

2. Rat long-term gavage study: Chadin *et al*., 1985, as cited in PHS 149, 1987. Groups of 41-45 CPJ rats (sex not specified) were administered HCBD by gavage at doses of 0.6, 5.8 or 37 mg/kg/day for 600 to 700 days. Forty-six animals served as vehicle controls and 90 as untreated controls. One out of 41 animals in the high-dose group and one out of 45 animals in the mid-dose group developed kidney tumors. No kidney tumors were observed in either the vehicle or untreated controls.

3. Mouse intraperitoneal injection study: Theiss *et al*., 1977. Two groups of male A/St mice received 12-13 intraperitoneal injections (3 times/week) of 4 or 8 mg HCBD/kg body weight in tricaprylin. All surviving mice (19 and 14 animals, respectively) were killed and examined for lung tumors 24 weeks after initiation of injections. The incidence of lung tumors was not increased compared with that of injected controls. IARC (1979) noted the limitations of a negative result obtained with this test system.

Other relevant data

HCBD was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when preincubated in the presence or absence of Arochlor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Haworth *et al*., 1983). HCBD was positive in the *Salmonella* assay when preincubated with rat liver microsomes and glutathione; the addition of rat kidney microsomes increased this activity. Reichert *et al*. (1984) and Green and Odum (1985) demonstrated the mutagenicity of HCBD metabolites in the bacterial mutation plate assay. The glutathione and cysteine conjugates of HCBD were shown to be direct acting mutagens in *Salmonella typhimurium* strain TA100; rat kidney microsomes markedly enhanced the mutagenic potency of the glutathione conjugate (Vamvakas *et al*., 1988). HCBD induced sister chromatid exchanges in Chinese hamster ovary cells in both the

presence and absence of Arochlor 1254-induced male Sprague-Dawley rat liver S9, but not chromosomal aberrations (Galloway *et al*., 1987). HCBD did not induce sex-linked recessive lethal mutations in *Drosophila* (NTP, 1991).

HCBD is a well-known kidney toxicant, and the nephrotoxocity has been linked to the formation of glutathione-conjugated metabolites, and beta lyase-dependent bioactivation of the glutathione conjugates (ATSDR, 1994). Kidney tumor induction by HCBD is postulated to be the result of the formation of reactive metabolites from glutathione conjugation (Henschler and Dekant, 1990). HCBD metabolites have been shown to covalently bind *in vivo* more readily to kidney DNA than to liver DNA (Schrenk and Dekant, 1989).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over HCBD exposure based on the induction of malignant kidney tumors in male rats and benign and malignant kidney tumors in female rats. Positive findings of genotoxicity, including evidence of mutagenicity and covalent binding to DNA at the target site add considerably to the level of concern.

There is a **HIGH** level of **concern over the extent of exposure** to HCBD, based on widespread human exposure in both the occupational and general environments. HCBD has been detected at low levels in air samples collected near chemical manufacturing facilities, in effluent water from U.S. chemical manufacturing facilities, in Mississippi River water, and in U.S. drinking water (IARC, 1979). According to the Toxics Release Inventory data for 1990, there is one chlorinated solvent manufacturing plant in California, with between 1000 to 9,999 pounds of the manufacturing by-product HCBD reported on-site (ATSDR, 1994). NTP estimated that between 3.3 to 6.6 million kg are produced annually in the U.S. (NTP, 1991).

References

Agency for Toxic Substances and Disease Registry (ATSDR, 1994). Toxicological Profile for hexachlorobutadiene. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin BH, Resnick MA, Anderson B, Zeiger E (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ Molec Mutagen* **10**(Suppl. 10):1-175.

Green T, Odum J (1985). Structure/activity studies of the nephrotoxic and mutagenic action of cysteine conjugates of chloro- and fluoroalkenes. *Chem.-Biol Interact* **54**(1): 15-31.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983). *Salmonella* mutagenicity test results for 250 chemicals. *Environ Mutagen* Suppl. **1**: 3-142.

Henschler D, Dekant W (1990). Nephrocarcinogenic xenobiotics. *Toxicol Lett* **53**:105-110.

International Agency for Research on Cancer (IARC, 1979). *IARC monographs on the evaluation of carcinogenic risk of chemicals to humans; Some halogenated hydrocarbons*. Volume 20, pp. 179-193. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of carcinogenic risk of chemicals to humansOverall evaluation of carcinogenicity: An updating of IARC monographs Volumes 1 to 42*. Supplement 7: p. 64. IARC, Lyon, France.

Kociba RJ, Keyes DG, Jersey GC, Ballard JJ, Dittenber DA, Quast JF, Wade CE, Humiston CG, Schwetz BA. (1977). Results of a two year chronic toxicity study with hexachlorobutadiene in rats. *Am Ind Hyg Assoc J* **38**:589-602.

National Institute for Occupational Safety and Health (NIOSH, 1994). NIOSH Pocket Guide to Chemical Hazards. U.S. Department of Health and Human Services. Center for Disease Control and Prevention. NIOSH.

National Toxicology Program (NTP, 1991). Toxicity Studies of hexachloro-1,3-butadiene in B6C3F1 mice (Feed Studies). NTP Tox 1. NIH Publication No. 91-3120. National Toxicology Program, Research Triangle Park, NC.

Public Health Service (PHS 149, 1987). U.S. Department of Health and Human Services. National Cancer Institute, Bethesda, MD.

Reichert D, Neudecker T, Schutz S (1984). Mutagenicity of hexachlorobutadiene, perchlorobutenoic acid and perchlorobutenoic acid chloride. *Mutat Res* **137(2-3)**: 89-93.

Schrenk D, Dekant W (1989). Covalent binding of hexachlorobutadiene metabolites to renal and hepatic mitochondrial DNA. *Carcinogenesis* **10(6)**: 1139-1141.

Theiss JC, Stoner GD, Shimkin MB, Weisburger EK (1977). Test for carcinogenicity of organic contaminates of United States drinking waters by pulmonary tumor response in strain A mice. *Cancer Res* **37**:2717-2720.

US Environmental Protection Agency (EPA, 1991). Integrated Risk Information System (IRIS).

Vamvakas S, Kordowich FJ, Dekant W, Neudecker T, Henschler D (1988). Mutagenicity of hexachloro-1,3-butadiene and its S-conjugates in the Ames test - Role of activation by the mercapturic acid pathway in its nephrocarcinogenicity. *Carcinogenesis* **9(6)**: 907-910.

CARCINOGENICITY DATA SUMMARY: LOVASTATIN

Lovastatin (mevinolin; CAS No. 7530-75-5) is used as a drug to decrease elevated serum total and low density lipoprotein cholesterol concentrations in the treatment of hyperchlolesterolemia. Lovastatin is marketed under the brand name Mevacor®. The recommended therapeutic dose range by the oral route is 0.2 to 1.6 mg/kg-day (MacDonald *et al.,* 1988).

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified in the published literature (RTECS, 1997).

Animal bioassays

1. Mouse 92-week oral studies: As cited by HSDB, 1997; FDA, 1995; MacDonald *et al.,* 1988. Groups of 50 male and 50 female mice were treated orally with 0 (2 groups), 20, 100, or 500 mg/kg-day of lovastatin for 92 weeks. Statistically significant increases of hepatocellular carcinoma were observed in the high-dose groups of both males (2/50, 6/50, and 19/50 for control I, control II, and high-dose males, respectively) and females (0/50, 0/50, and 7/50 for control I, control II, and high-dose females, respectively) ($p<0.001$ for high-dose females). There was a statistically significant increase in the incidence of pulmonary adenomas in high-dose female mice ($p<0.02$) and a dose-related increase in forestomach papillomas in female mice given oral lovastatin doses of 100 or 500 mg/kg-day. In addition, the incidence rates of forestomach papillomas in the mid- and high-dose females were also significantly higher than that of the controls ($p<0.02$).

2. Rat 2-year oral studies: HSDB, 1997; FDA, 1995; MacDonald *et al.*, 1988. Groups of 50 male and 50 female rats were treated with 0, 0, 5, 30, or 180 mg/kg-day of lovastatin for two years. A statistically significant increase in hepatocellular carcinoma was observed in the high-dose males (0/50, 0/50, and 3/50 for control I, control II, and high-dose males, respectively) ($p=0.03$). No excess tumor incidences were observed among the female rats exposed to lovastatin.

Other relevant data

Lovastatin did not exhibit mutagenic potential in microbial systems (Ames test) with or without metabolic activation. It was negative in several *in vitro* mammalian cell systems (rat or mouse hepatocytes, Chinese hamster ovary cell, V-79 cell forward mutation study), and *in vivo* in mouse bone marrow chromosomal aberration studies (HSDB, 1997). Lovastatin is hydrolyzed to mevinolinic acid, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme essential in cholesterol biosynthesis. A product of HMG-CoA reductase is mevalonic acid which has been shown to be essential for DNA synthesis. There is some *in vitro* evidence that lovastatin can inhibit DNA synthesis during the S-phase of the cell life cycle through inhibition of HMG-CoA reductase (HSDB, 1997). A number of other HMG-CoA reductase inhibitors such as pravastatin, simvastatin, and fluvastatin have also been shown to cause liver or forestomach tumors in rodents.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** as lovastatin has been shown to induce hepatocellular carcinoma in male rats and in mice of both sexes. The level of concern is supported by statistically significant increases in the incidence of pulmonary adenomas in high-dose female mice, a dose-related increase in benign forestomach tumors in female mice, and by the observation that other HMG-CoA reductase inhibitors also induce liver or forestomach tumors in rodents.

There is a **HIGH** level of **concern over the extent of exposure** as lovastatin is widely prescribed to patients to control high cholesterol levels.

References

Food and Drug Administration (FDA, 1995). A request of carcinogenicity study for Lovastatin under the Freedom of Information Act to the Food and Drug Administration, Division of Federal-State Relations, Rockville, MD.

Hazardous Substances Data Bank (HSDB, 1997). National Library of Medicine. Bethesda, MD.

MacDonald JS, Gerson RJ, Kornbrust DJ, Kloss MW, Prahalada S, Berry PH, Alberts AW, Bokelman DL. (1988) Preclinical evaluation of lovastatin. *Am J Cardiol* **62**:16J-27J.

Physician's Desk Reference (PDR, 1997). Mevacor® Tablets. Medical Economics Company, Inc. Montvale, NJ. p. 1742.

Registry of Toxic Effects of Chemical Substances (RTECS, 1997). Database produced by the U.S. National Institute for Occupational Safety and Health. Version date 1/97.

CARCINOGENICITY DATA SUMMARY: METHYLPHENIDATE AND ITS HYDROCHLORIDE (RITALIN®)

Methylphenidate hydrochloride (Ritalin®; methyl α-phenyl-α-(2-piperidyl)acetate; CAS No. 298-59-9; CAS No. for methylphenidate is 113-45-1) is a drug used to treat narcolepsy and attention deficit hyperactivity disorders (ADHD). A derivative of piperidine, methylphenidate was first marketed in the 1960s (Diller, 1996). Ninety percent of the current total is made by Ciba-Geigy and is used in the United States (Leutwyler, 1996). It is a mild central nervous system stimulant, although its mode of action is not completely understood.

Records of production quotas maintained by the Drug Enforcement Agency show a steady output of approximately 1700 kilograms of legal methylphenidate HCl through the 1980's followed by a sharp increase in production in 1991 (Diller, 1996). From 1990 to May 1995, the annual US production of methylphenidate increased by 500% to 10,410 kilograms (Diller, 1996). Data collected in 1993 found that ~72% of the 1.8 million persons receiving medication for ADHD were taking methylphenidate HCl (Diller, 1996). Ritalin® production levels (most recent data from 1995) suggest that 2.6 million people were taking Ritalin®, the vast majority of whom were children ages 5-12 (Diller, 1996). In comparison, according to the US Census Bureau in 1994 there were 260,341,000 people in the US, with 45,165,682 of them being children from the ages of 5 to 17 (Byerly and Deardorff, 1995). These data illustrate that almost 1% of the entire US population is taking Ritalin®. When looking at children alone this percentage is even higher. Furthermore, methylphenidate HCl also has abuse potential, especially among teenagers (Diller, 1996).

The average daily dosage of methylphenidate HCl for medical treatment is 10 mg, 2 or 3 times daily, with dosages above 60 mg and treatment below the age of five not recommended (PDR, 1994). The usual child dosage is 5 mg, twice daily (0.3-1 mg/kg). However, dosages are individualized according to the needs and responses of the patient, as well as on the basis of factors such as age and body weight. Exposure to methylphenidate can therefore vary from person to person. The average duration of treatment is 2 years when prescribed to elementary school-age children, 4 years for middle school-age children, and 7 years for high school-age children (NTP, 1995).

The US FDA has reported that the NTP study produced a 'weak signal' of cancer-causing potential (US FDA, 1996). US FDA has indicated that as a follow-up, further short-term *in vivo* studies will be conducted, including: carcinogenicity in the neonatal mouse model, carcinogenicity in the P53 mouse model, metabolism and exposure studies, and 'possibly a repeat of the mouse lymphoma study'. US FDA-sponsored epidemiological follow-up will include a case-control study comparing "the odds of prior exposure to Ritalin® in patients with hepatoblastoma with that in control subjects" and an evaluation of the feasibility of performing a cohort study in Ritalin-exposed children examining multiple cancer endpoints.

Carcinogenicity Data available:

Epidemiological studies

In a screening study of participants in a health plan in Oakland, California, taking a wide range of prescription drugs between 1969 and 1973 and followed up 1984 (11-15 years later), 529 study subjects had received methylphenidate HCl (Selby *et al.*, 1989). No increased risk of cancer at any site was associated with methylphenidate HCl exposure. Fifteen cases of cancer were observed (32.7 cases expected), resulting in a standardized mortality ratio of 0.46 (negative association with $p<0.002$). Results were not adjusted for confounding due to smoking or alcohol consumption because of limited data available for the cohort. Occurrence of cancer in the general population was drawn from the California Resource for Cancer Epidemiology, the San Francisco Bay Area tumor registry, and the hospital's discharge abstracts.

Animal bioassays

1. Rat long-term feeding studies: NTP, 1995. F344 rats (70/sex/dose) were fed 0, 100, 500, or 1,000 ppm methylphenidate HCl for up to two years. No significant increases in chemical-related neoplasm incidences were observed in male or female rats. The NTP concluded that there was no evidence of carcinogenic activity for methylphenidate hydrochloride in male or female rats under these conditions.

2. Mouse long-term feeding studies: NTP, 1995. B6C3F$_1$ mice (70/sex/dose) were fed 0, 50, 250, or 500 ppm methylphenidate HCl for 2 years. An increased incidence of hepatoblastoma occurred in high-dose male mice (0/50, 1/50, 1/50, 5/50; p=0.028) and increased incidences of hepatocellular adenoma occurred in both high-dose males (18/50, 18/50, 16/50, 29/50; p=0.02) and females (6/49, 10/48, 10/49, 28/50; p<0.01). The incidences of hepatocellular carcinoma were similar among control and exposed mice. The increase in combined hepatocellular adenoma, carcinoma and hepatoblastoma was significant in high-dose males (p=0.037) (24/50, 23/50, 26/50, 34/50). Combined hepatocellular adenoma and carcinoma was also significantly increased in high-dose females (p<0.001) (9/49, 11/48, 11/49, 30/50). The incidences of eosinophilic foci were increased in 500 ppm methylphenidate HCl-dosed male mice (6/50; 8/50; 9/50; 14/50) and female mice (3/49, 3/48, 8/49, 25/50). Based on the occurrence of hepatocellular neoplasms, the NTP concluded that there was some evidence of carcinogenic activity for methylphenidate HCl in male and female B6C3F$_1$ mice.

Other relevant data

Methylphenidate caused small, but significant, increases in the frequency of sister chromatid exchanges in lymphocytes from pediatric patients (Walker and Dumars, 1977). This result was only presented in abstract form and not followed-up in the peer-reviewed literature. Methylphenidate HCl was not mutagenic in *Salmonella typhimurium* with and without metabolic activation with S-9 (NTP, 1995) and it did not induce transformation of F344 rat embryo cells (Price *et al.*, 1978). It did, however, increase the frequency of sister chromatid exchanges and chromosomal aberrations in CHO cells (NTP, 1995). Methylphenidate was found to be non-mutagenic in L5178Y mouse lymphoma cells both with and without metabolic activation with S-9 (Rudd *et al.*, 1983) and did not induce unscheduled DNA synthesis when incubated with F344 male rat hepatocytes and tritiated thymidine (Mirsalis *et al.*, 1983).

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH** level of **carcinogenicity concern over** methylphenidate and its hydrochloride due to the hepatocarcinogenic activity seen in male and female B6C3F$_1$ mice. A significant increase in the incidence of a rare malignant liver tumor type (hepatoblastoma) in male mice contributes to this level of concern. Concern is mitigated by the fact that the majority of the treatment-related hepatocellular tumors (as opposed to hepatoblastomas) were non-malignant tumors. There was also some evidence of genotoxicity in experimental animals with positive findings of chromosome aberration and increased SCE in exposed cells.

There is a **HIGH** level of **concern over the extent of exposure** since methylphenidate HCl is a commonly prescribed drug given on a long-term basis to children and others with attention deficit hyperactivity disorders and narcolepsy.

References

Byerly ER, Deardorff K (1995). National and State Population Estimates: 1990 to 1994, US Bureau of the Census, Current Population Reports, P25-1127, US Government Printing Office, Washington, DC, 1995.

Diller LH (1996). The run on Ritalin: Attention deficit disorder and stimulant treatment in the 1990s. *Hastings Center Report* **26**:12-18.

Dunnick JK, Elwell MR, Haseman JK (1996). Decreased incidence of spontaneous mammary gland neoplasms in female F344 rats treated with amphetamine, methylphenidate, or codeine. *Cancer Lett* **102**(1-2): 77-83.

Dunnick JK, Hailey JR (1995). Experimental studies on the long-term effects of methylphenidate hydrochloride. *Toxicology* **103**(2): 77-84.

Leutwyler K (1996). Paying attention: The controversy over ADHD and the drug Ritalin is obscuring a real look at the disorder and its underpinnings. *Sci Amer* **275**:12;14.

Mirsalis J, Tyson K, Beck J, Loh F, Streinmetz K, Contreras C, Austere L, Martin S, Spalding J (1983). Induction of unscheduled DNA synthesis (UDS) in hepatocytes following in vitro and in vivo treatment [Abstract]. *Environ Mutagen* **5**:482.

National Toxicology Program (NTP, 1995). Toxicology and carcinogenesis studies of methylphenidate hydrochloride (CAS No. 298-59-9) in F344 rats and B6C3F_1 mice (feed studies). NTP Technical Report Series No. 439, NIH Publication No. 95-3355.

Physician's Desk Reference (PDR, 1994). Methylphenidate Hydrochloride. p. 897

Price PJ, Gregory EA, Skeen PC (1978). Ritalin, benzedrine and dexedrine do not transform F 1706 rat cells. *Cancer Lett* **5**:345-349.

Rudd CJ, Mitchell AD, Spalding J (1983). L5178Y mouse lymphoma cell mutagenesis assay of coded chemicals incorporation analyses of the colony size distribution [Abstract]. *Environ Mutagen* **5**:419.

Selby JV, Friedman GD, Fireman BH (1989). Screening prescription drugs for possible carcinogenicity: Eleven to fifteen years of follow-up. *Cancer Res* **49**:5736-5747.

US Food and Drug Administration (US FDA, 1996). Ritalin: FDA Talk Paper T96-4, Jan 12, 1996.

Walker AP, Dumars KW (1977). Commonly used pediatric drugs, sister chromatid exchanges and the cell cycle [Abstract]. *Amer J Hum Genet* **29**:110A.

CARCINOGENICITY DATA SUMMARY: 4-METHYLQUINOLINE

4-Methylquinoline (lepidine; CAS No. 491-35-0) is a contaminant associated with the use of hydrocarbons in both shale oil and coal gasification and wood treatment processes. Contamination has been reported in groundwater and soil samples taken near these processes. NIOSH in its 1983 National Exposure Survey estimated that 1557 employees in the U.S. were potentially exposed (RTECS, 1997). In addition, 4-methylquinoline is a component of cigarette smoke (Adams *et al.,* 1983) and urban particulate matter (Dong *et al.,* 1977).

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were found in the literature.

Animal bioassays

1. Mouse i.p. injection studies: LaVoie *et al.,* 1988. Newborn CD-1 mice, 29 females and 28 males, were treated with 3 intraperitoneal injections containing 0.25, 0.5 and 1.0 µmol 4-methylquinoline in DMSO on days 1, 8 and 15 of life, respectively, then observed for 1 year. In male mice, an increased incidence of liver tumors (23/28) (20 adenomas, 3 hepatomas) in the treated group was observed compared with 4/21 in the vehicle control mice. No increased incidence of tumors was observed for female mice (0/29 in the treated and 0/21 in the controls).

2. Rat suprascapular injection studies: LaVoie *et al.,* 1988. Newborn Sprague-Dawley rats, 26 males and 20 females, were administered suprascapular injections containing 200 µmol/kg body weight in DMSO on day 1 of life, then 100 µmol/kg-bodyweight weekly for weeks 2-7, followed by 200 µmol/kg-body weight at week 8, followed by observation for 70 weeks. No significant increase in tumors was observed in the male or female rats.

3. Mouse skin painting study: LaVoie *et al.,* 1984. 4-Methylquinoline in acetone was painted on the backs of 29 female Sencar mice 10 times over 20 days (total dose 7.5 mg) and promoted with 2 µL tetradecanoyl phorbol acetate twice weekly for 18 weeks. Skin tumors were observed in 45% of treated animals compared to 7.5% in the control animals.

Other relevant data

4-Methylquinoline was found to be a strong mutagen in *Salmonella* mutagenicity assays compared to quinoline and isomeric methylquinolines (Saeki *et al.,* 1996). 4-Methylquinoline was mutagenic in *S. typhimurium* strains TA 98 and TA100 with exogenous activation (+S9) but not without (-S9) (Nagao *et al.,* 1977) and caused unscheduled DNA synthesis in rat hepatocytes (LaVoie *et al.,* 1991). The structurally-related compound quinoline is listed as known to the State to cause cancer under Proposition 65, based on a liver tumor response in rodents.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over 4-methylquinoline. This is associated with the increased incidence of liver tumors in newborn male mice (after only three intraperitoneal injections), and the initiation of tumors in a skin painting study in female mice. 4-Methylquinoline was observed to be genotoxic in several assays. Increased carcinogenicity concern stems from its structural similarity to quinoline, a compound already listed as known to cause cancer on the Proposition 65 list, which produced tumors at the same site as 4-methylquinoline.

There is a **HIGH** level of **concern over the extent of exposure** to 4-methylquinoline because it is a component of tobacco smoke, is associated with organic portions of urban particulate matter, and has been found as a contaminant of groundwater and soil.

References

Adams JD, LaVoie EJ, Shigematsu A, Owens P, Hoffmann D (1983). Quinoline and methylquinolines in cigarette smoke: comparative data and the effect of filtration. *J Anal Toxicol* **7**:293-296.

Dong M, Lock DC, Hoffmann D (1977). Characterization of aza-arenes in basic organic portion of suspended particulate matter. *Environ Sci Technol* **11**:612-618.

LaVoie EJ, Shigematsu A, Adams EA, Rigotty J, Hoffmann D (1984). Tumor-initiating activity of quinoline and methylated quinolines on the skin of Sencar mice. *Cancer Lett* **22**:269-273.

LaVoie EJ, Dolan S, Little P, Wang C-X, Sugie WS, Rivenson A (1988). Carcinogenicity of quinoline, 4- and 8-methylquinoline and benzoquinolines in newborn mice and rats. *Food Chem Toxicol* **26**(7):625-629.

LaVoie EJ, Defauw J, Fealy M, Way BM, McQueen CA (1991). Genotoxicity of fluoroquinolines and methylquinolines. *Carcinogenesis* **12**(2):217-220.

Nagao M, Yahagi T, Seino Y, Sugimura T, Ito N (1977). Mutagenicities of quinoline and its derivatives. *Mutat Res* **42**(3):335-342.

Registry of Toxic Effects of Chemical Substances (RTECS, 1997). Database produced by the U.S. National Institute for Occupational Safety and Health. Version date 1/97.

Saeki K, Takahashi K, Kawazoe Y (1996). Potent mutagenic potential of 4-methylquinoline: metabolic and mechanistic considerations. *Biol Pharm Bull* **19**(4):541-546.

CARCINOGENICITY DATA SUMMARY: MX (3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2[5H]-FURANONE)

MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone; CAS No. 77439-76-0) is a chemical by-product of chlorination of drinking water. It is produced when chlorine reacts with organic materials, including humic acids, in water. MX has been detected in drinking water in the U.S. with levels ranging from 3 to 67 parts per trillion (Melnick *et al.,* 1997).

Carcinogenicity Data available:

Epidemiological studies

Several epidemiological studies have suggested that consumption of chlorinated drinking water may be associated with increases of some forms of cancer in humans (Morris, 1995).

Animal bioassays

1. Rat 104-week drinking water studies: Komulainen *et al.,* 1997. Groups of 50 Wistar rats of each sex were administered MX in drinking water for 104 weeks at concentrations yielding average daily doses of 0.4 mg/kg (low-dose), 1.3 mg/kg (mid-dose), and 5.0 mg/kg (high-dose) for males and 0.6 mg/kg, 1.9 mg/kg, and 6.6 mg/kg for females, respectively. Fifty male and female rats served as controls. Among the male rats, statistically significant dose-dependent increases in follicular carcinoma ($p<0.0001$) and adenoma ($p=0.0045$) of the thyroid gland, cortical adenoma ($p=0.0001$), cholangioma ($p=0.0009$) and adenoma ($p=0.0142$) of the liver, Langerhan's cell adenomas ($p=0.0116$) of the pancreas, alveolar and bronchiolar adenomas ($p=0.0015$), and basal cell tumors ($p=0.0314$) of the skin were observed. Similar tumor responses were seen in the female rats: dose-related trends were reported for follicular carcinoma ($p<0.0001$) and adenoma ($p<0.0001$) of the thyroid gland, cortical adenoma ($p=0.0098$), cholangioma ($p<0.0001$) and adenoma ($p<0.0001$) of the liver, lymphoma and leukemia ($p=0.0474$), and adenocarcinoma ($p=0.0012$) and fibroadenoma ($p=0.0090$) of the mammary gland. Komulainen *et al.,* noted that MX is a potent carcinogen in both male and female rats, increasing the risk of cancer even at the lowest dose studied and at doses not overtly toxic to rats.

Other relevant data

Oral administration of MX in the water to rats resulted in a dose-related increase of cell proliferation and lipid peroxidation in the gastric mucosa (Nishikawa *et al.,* 1994). Over 30 studies, noted in the published literature, indicate that MX has genotoxic potential. MX caused mutations in bacteria and mammalian cells, micronuclei, sister chromatid exchanges, DNA strand breaks, alkali-labile sites, unscheduled DNA synthesis, and formed DNA and protein adducts.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over MX since administration of the chemical in drinking water produced a dose-related increase of liver and thyroid cancers in male and female rats. Significant increases in tumors of the adrenal glands, lungs, pancreas, mammary glands, and lymphomas and leukemias were also observed in the high-dose animals. Additional concern comes from extensive evidence demonstrating MX's genotoxic potential.

There is a **HIGH** level of **concern over the extent of exposure**. MX is a by-product of the chlorination of drinking water. As water chlorination is used by many water utilities in California, exposure of the general population to this water contaminant is potentially widespread.

References

Komulainen H, Kosma V, Vaittinen S, Vartiainen T, Kaliste-Korhonen E, Lötjönen S, Tumominen RK, Tuomisto J (1997). Carcinogenicity of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone in the rat. *J Natl Cancer Inst* **89**(12):848-856.

Melnick RL, Boorman GA, Dellarco V (1997). Water chlorination, 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX), and potential cancer risk. *J Natl Cancer Inst* **89** (12):832-833.

Morris RD (1995). Drinking water and cancer. *Environ Health Perspect* **103**:225-231.

Nishikawa A, Kinae N, Furukawa F, Mitsui M, Enami T, Hasegawa T, Takahashi M (1994). Enhancing effects of 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX) on cell proliferation and lipid peroxidation in the rat gastric mucosa. *Cancer Lett* **85**(2):151-157.

CARCINOGENICITY DATA SUMMARY: PHENELZINE AND ITS ACID SALTS

Phenelzine (β-phenylethyl hydrazine, 2-phenylethyl hydrazine; CAS No. 156-51-4) is used to produce phenelzine sulfate (CAS No. 51-71-8), which is used solely as a prescription drug in the U.S. It acts as a monoamine oxidase inhibitor and is used in the treatment of certain types of depression and in certain phobic anxiety states. It is first given in a daily dose of 30-45 mg and then adjusted to 15-75 mg after the first two weeks (IARC, 1980). Only one company in the U.S. is believed to produce this drug. Phenelzine is used only in the preparation of the hydrochloride and sulfate salts (IARC, 1980). In 1978, U.S. imports of phenelzine sulfate through the principal U.S. customs districts amounted to 450 kg (IARC, 1980). Based upon limited evidence of carcinogenicity in experimental animals and inadequate evidence of carcinogenicity in humans, IARC (1980; 1987) has classified phenelzine sulfate as a Group 3 carcinogen. However, an evaluation using more recent versions of the inference guidelines used by IARC and other authorities might place more reliance on the observations of genetic toxicity, the structural analogy with several compounds known to cause cancer, and the case report of possible human carcinogenicity (see below).

Carcinogenicity Data available:

Epidemiological studies

A case report described a 64-year old woman treated with phenelzine for 6 years who developed angiosarcoma of the liver, multiple peritoneal angiosarcomas, and an osteolytic lesion in the humerus suggestive of metastatic disease (Daneshmend *et al.*, 1979; IARC, 1980). The patient had no history of exposure to other compounds suspected in the development of angiosarcomas (thorium dioxide, arsenic, vinyl chloride) and had been medicated only occasionally with diazepam.

Animal bioassays

1. Mouse lifetime drinking water studies: Toth and Shimizu, 1974; Toth, 1976; Toth and Nagel, 1976. Groups of 50 male and 50 female Swiss mice were given drinking water containing 0.015% phenelzine sulfate for a lifetime. The control groups consisted of 100 male and 100 female mice. In treated female mice, the incidence of lung adenomas or adenocarcinomas (combined) was significantly increased when compared to the incidence in controls (28/50 *vs.* 21/100: $p<0.001$), and the incidence of angiomas and angiosarcomas (combined) was also increased (22/50 *vs.* 5/100: $p<0.001$). In treated male mice, there was an increase in the incidence of lung adenomas or adenocarcinomas (combined) above the incidence in controls, but it was just short of being statistically significant (18/50 *vs.* 23/100: $p=0.07$).

Other relevant data

Male Sprague-Dawley rats fed diets containing phenelzine for 87 weeks and co-treated with subcutaneous injections of 1,2-dimethylhydrazine did not develop significantly increased intestinal adenocarcinomas over control animals, suggesting phenelzine is not co-carcinogenic (Gershbein and Rao, 1992).

In the absence of metabolic activation, phenelzine sulfate was mutagenic to *Salmonella typhimurium* strain TA100 (IARC, 1980; citing Shimizu *et al.*, 1978). Phenelzine sulfate has also been shown to inactivate *Bacillus subtilis* transforming DNA (IARC, 1980; citing Freese *et al.*, 1968) and to be reactive with DNA in the *pol* A^+/A^- test in *Escherichia coli* (IARC, 1980; citing Rosenkranz and Carr, 1971). Phenelzine was not found to damage DNA by the alkaline elution technique in liver and lung tissues of intraperitoneally-treated Swiss mice (Parodi *et al.*, 1981).

Phenelzine is structurally related to hydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, 1,2-diethylhydrazine and 1,2-diphenylhydrazine, all of which are listed under Proposition 65 as substances known to the State to cause cancer.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over phenelzine sulfate because it caused statistically significant increases in the incidences of malignant and benign lung tumors, and hemangiomas or hemangiosarcomas, in female

mice. An increase in the incidence of malignant and benign lung tumors in male mice, although just short of statistical significance, adds to the concern, as do the observations of genotoxicity in bacterial test systems, and structural analogies with several known carcinogens.

There is a **HIGH** level of **concern over the extent of exposure** to phenelzine sulfate. From the data on the amount imported, it appears that this drug may be prescribed to thousands of patients in the U.S. each year for relatively common disorders found in the general population. The daily dose prescribed to patients (e.g., 75 mg/day) may be equal to several percent of the dose that produced cancer in mice, and the drug may be administered to individuals on a long-term basis.

References

Daneshmend TK, Scott GL, Bradfield JWB (1979). Angiosarcoma of liver associated with phenelzine. *Br J Med* **i**:1679.

Freese E, Sklarow S, Bautz Freese E (1968). DNA damage caused by antidepressant hydrazines and related drugs. *Mutat Res* **5**:343-8.

Gershbein LL, Rao KC (1992). Action of hydrazine drugs in tumor-free and 1,2-dimethylhydrazine-treated male rats. *Oncol Res* **4**(3):121-7.

International Agency for Research on Cancer (IARC, 1980*). IARC Monographs on the Carcinogenic Risk to Humans, Some Pharmaceutical Drugs*. Volume 24, pp. 175-84. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of carcinogenic risks to humans; Overall evaluation of carcinogenicity: An updating of IARC monographs Volumes 1 to 42*. Supplement 7, pp. 312-313. IARC, Lyon, France.

Parodi S, De Flora S, Cavanna M, Pino A, Robbiano L, Bennicelli C, Brambilla G (1981). DNA-damaging activity *in vivo* and bacterial mutagenicity of sixteen hydrazine derivatives as related quantitatively to their carcinogenicity. *Cancer Res* **41**(4):1469-82.

Rosenkranz HS, Carr HS (1971). Hydrazine antidepressants and isoniazid: potential carcinogens. *Lancet* **i**:1354-5.

Shimizu H, Hayashi K, Takemura N (1978). Relationships between the mutagenic and carcinogenic effects of hydrazine derivatives (Jpn). *Jpn J Hyg* **33**:474-85.

Toth B, Shimizu H (1974). 1-Carbamyl-2-phenylhydrazine tumorigenesis in Swiss mice. Morphology of lung adenomas. *J Natl Cancer Inst* **52**:241-51.

Toth B (1976). Tumorigenicity of β-phenylethylhydrazine sulfate in rats. *Cancer Res* **36**:917-21.

Toth B, Nagel D (1976). Tumorigenesis investigations with β-phenylethylhydrazine sulfate, phenylhydrazine hydrochloride, and ethylhydrazine hydrochloride in mice (Abstract no. 41). *Am J Pathol* **82**:40a-41a.

CARCINOGENICITY DATA SUMMARY: PYRROLIZIDINE ALKALOIDS WHICH ARE METABOLIZED TO DEHYDRORETRONECINE OR DEHYDROHELIOTRIDINE

Pyrrolizidine alkaloids occur in a wide variety of plants with worldwide distribution. Many of these alkaloids appear to have a common mechanism of tumor pathogenesis which involves metabolism in the liver to the putative toxic metabolites, dehydroretronecine, or its enantiomer, dehyroheliotridine [(+/-) 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine]. These metabolites have been shown to have potent antimitotic potential, to alkylate DNA and other cellular targets, to cross-link DNA, and to be demonstrably hepatotoxic. In this data summary, OEHHA has grouped together, as a class listing, those pyrrolizidine alkaloids functioning through this common carcinogenic mode of action. The data summary covers jacobine, hydroxysenkirkine, istatidine, lasiocarpine, monocrotaline, petasitenine, retrorsine, riddelliIne, seneciphylline, senkirkine, and symphytine, and the metabolites dehydroretronecine and dehydroheliotridine.

Dehydroretronecine (CAS No. 23107-12-2) is a major water soluble metabolite of the plant pyrrolizidine alkaloids, jacobine (CAS No. 471-14-7), hydroxysenkirkine (CAS No. 26782-43-4), monocrotaline (CAS No. 315-22-0), retrorsine (CAS No. 480-54-6), riddelliine (CAS No. 23246-96-0), seneciphylline (CAS No. 480-81-9), and senkirkine (CAS No. 6882-01-5) (IARC, 1976; IARC 1983). Dehydroheliotridine (CAS No. 26400-24-8) is a major water soluble metabolite of lasiocarpine (CAS No 303-34-4) (IARC 1976), and presumably other esters of heliotridine such as petasitenine (CAS No. 60102-37-6), and symphytine (CAS No. 2257195-5) (IARC, 1976; IARC 1983). Istatidine (CAS No. 15503-86-3) forms dehydroretronecine through indirect metabolic pathways involving alkaloid-N-oxides (IARC 1976, Couet *et al.* 1996). There is also some evidence that microsomes transform these pyrrolizidine alkaloids to a racemic mixture (+/-) rather than pure enantiomers of dehydroretronecine (-) and dehyroheliotridine (+) (Kedzierski and Buhler, 1985).

A significant potential for human exposure to these compounds comes from consumption of pyrrolizidine alkaloid-containing plants used as herbal remedies, teas and in foods. An estimated 60 plants containing pyrrolizidine alkaloids are used as medicinal substances or as teas, including comfrey root and leaf (*Symphytum* spp.) and coltsfoot leaf and flower (*Tussilago farfara*) which are commonly used in the U.S. (AHPA, 1997). One pyrrolizidine alkaloid, monocrotaline, is present in seeds and vegetation of the leguminous plant *Crotalaria spectabilis*, and has been implicated in a number of diseases of the liver in various populations around the world (Shumaker *et al*., 1976). Dehydroretronecine is a major metabolite of pyrrolizidine alkaloids found in the plants *Senecios jacobaea* and *Senecios vulgaris*. These two plants are responsible for many cases of livestock poisoning in the western U.S., and the alkaloids from these plants have been found in the milk of cows and goats and in some samples of honey (Reed *et al.*, 1988). *Petasites japonicus*, a form of coltsfoot containing petasitenine and senkirkine, is reported to be used as an herbal remedy (cough remedy, expectorant) and food in Japan (IARC, 1976; IARC, 1983). Monocrotaline and lascioscapine are listed on the Proposition 65 list of carcinogens, and are classified by IARC (1987) as Group 2B carcinogens, based on sufficient evidence in animals. IARC (1987) lists istatidine, petasitenine, retrorsine and senkirkine as Group 3, based on limited evidence in animals; jacobine, riddelliine, and hydroxysenkirkine are listed as Group 3, based on insufficient evidence in animals; and seneciphylline is listed as Group 3, based on no adequate data. (IARC considered the compounds individually, rather than as a related group, with their metabolites.)

Carcinogenicity Data available:

Epidemiological studies

No human carcinogenicity studies were found in the literature, although there are a number of documented cases of liver damage or death from ingestion of pyrrolizidine alkaloid-containing plants or plant extracts (KAI 1995).

Animal bioassays

A number of studies have been conducted with dehydroretronecine or its enantiomer dehydroheliotridine, the common metabolite of several naturally occurring plant pyrrolizidine alkaloids. They are briefly described below.

Dehydroretronecine studies

1. Rat long-term s.c. injection study: Allen *et al.,* 1975. Sixty male Sprague-Dawley rats were injected subcutaneously with dehydroretronecine (in saline), at a dose of 20 mg/kg-body weight biweekly for 4 months and then with 10 mg/kg-body weight for the following 8 months. A separate group of rats were treated with the parent pyrrolizidine alkaloid, monocrotaline, at a dose of 5 mg/kg-body weight subcutaneously injected every other week for 12 months. Rats were observed for an additional 10 months. Control rats received biweekly injections of saline. Rats treated with dehydroretronecine exhibited rhabdomyosarcomas (31/60) and rhabdomyosarcoma with metastases (5/60). Rats treated with monocrotaline exhibited rhabdomyosarcomas (2/60), hepatocellular carcinoma (2/60), acute myelogenous leukemia (2/60) and pulmonary adenoma (2/60). Data from the control rats were not presented.

2. Rat long-term s.c. injection study: Shumaker *et al.,* 1976. Male Sprague-Dawley rats were divided into two treatment groups (60 animals/group) and one control group (45 animals). One experimental group received dehydroretronecine at a dose of 20 mg/kg-body weight by subcutaneously injection (in saline) every other week for 4 months followed by injections at a dose of 10 mg/kg-body weight for the succeeding 8 months. The second experimental group received the parent pyrrolizidine alkaloid, monocrotaline at doses of 5 mg/kg-body weight subcutaneously injections every other week for 12 months. All animals were followed for an additional 12 months after cessation of exposure. The dehydroretronecine-treated rats exhibited an increased incidence in rhabdomyosarcomas (39/60), 5 of which showed metastases. The monocrotaline treated rats exhibited an overall tumor incidence of 31/60 which was widely distributed over various tumor types (e.g., lung, liver, leukemia, rhabdomyosarcoma, adrenal). The only tumors reported for the control rats were 2/45 adrenal adenomas. The authors attributed the variation in tumor specificity between dehydroretronecine and its parent compound, monocrotaline, to the fact that monocrotaline needs to be metabolized to its proximate carcinogen before an effect can be realized.

3. Mouse long-term dermal absorption study: Mattocks and Cabral, 1982. Female LACA mice received 47 weekly topical applications of 5 μmol dehydroretronecine (in acetone) and then were observed for 55 weeks. Skin tumors (malignant carcinomas and sarcomas) were observed in 5/20 treated mice compared to 0/19 vehicle controls ($p<0.02$).

4. Mouse study (a) dermal absorption, (b) *s.c.* injection, (c) dermal absorption/s.c. injection combined: Johnson *et al.,* 1978. Female STS Swiss mice (8 weeks old,) were divided into one control group of 15 animals and experimental groups I, II and III, comprised of 25, 25 and 75 mice, respectively. Mice in group I were administered dehydroretronecine topically (20 mg/kg-body weight in acetone). Group II received subcutaneous injections (20 mg/kg-body weight in saline), and group III received a topical application (20 mg/kg-body weight in acetone) and a subcutaneous injection (20 mg/kg-body weight in saline). Doses were administered once per week for 4 weeks (or for 6 weeks if no tumors appeared at 4 weeks). Mice were then observed for 15 months. An increased incidence of skin tumors (primarily basal cell and squamous cell carcinomas) relative to controls was observed at the site of application: controls (0/11), group I (6/16), group II (13/21), and group III (28/55). Metastases to lung, liver and/or spleen were observed in 4 of the mice in group II and 8 of the mice in group III.

Dehydroheliotridine studies

5. Rat i.p. injection study: Peterson *et al.* 1983. Rats of the "Hooded" strain were divided into 24 animals per group and "9 injections were given over 32 weeks" beginning at 10 weeks of age. Group 1 was given weekly injections of 60 mg/kg body weight of thioacetamine (a mitosis stimulating agent). Group 2 was administered dehydroheliotridine once every 4 weeks, beginning at 10 weeks of age: the first dose was 76.5 mg/kg body weight, the second was 65 mg/kg, and the reminder was 60 mg/kg. A total of "9 injections were given over 32 weeks". Group 3 was given both thioacetamine and dehydroheliotridine following the same regimen as Groups 1 and 2. Group 4, the control group, was injected with saline. The lifespan of the rats was significantly shorted in the dehyroheliotridine- or combined dehydroheliotridine / thioacetamine-treated groups. The authors reported a statistically significant increase in malignant tumors (total tumors) relative to controls for both the dehyroheliotridine- or dehydroheliotridine/thioacetamine-treated groups. Tumors in the dehyroheliotridine-treated animals were distributed over many tissues, including the abdomen, thorax, pancreas, adrenal gland,

lung, abdominal wall, duodenum, cecum, forebrain, liver, and ileum. It should be noted that many of these tumors (fibrosarcomas and carcinomas) were located in the abdominal cavity or on the intestines the near the site of administration of dehydroheliotridine.

A number of carcinogenicity studies have been conducted with the parent pyrrolizidine alkaloids (e.g., hydroxysenkirkine, isatidine, lasciocarpine; monocrotaline, petasitenine, retrorsine, or riddelliine) resulting in increased incidence of tumors or early carcinogenic effects (IARC, 1976; IARC 1983). The following are brief descriptions of these studies.

6. Rat long-term drinking water study: Schoental *et al.*, 1954, as reported by IARC, 1976. Ten male and 4 female Wistar rats were given retrorsine via drinking water (0.03 mg/ml) 3 days per week for life. Six male rats showed nodular hyperplasia of which 4 were confirmed as hepatomas. An additional male had a hemorrhagic tumor of the liver and one lung adenoma was observed in one female. Information on control animals was not described in the IARC review.

7. Rat long-term drinking water study: Schoental *et al.*, 1954, as reported by IARC, 1976. 22 albino Wistar rats (8 male, 14 female) were treated with istatidine in drinking water (0.05 to 0.03 mg/mL) 3 days/week for approximately 20 months. Of 7 rats (3 male, 4 female) surviving 14-21 months of treatment, 6 had nodular hyperplasia of the liver: hepatomas were observed in the 4 surviving female rats. Information on control animals was not described in the IARC review.

8. Rat drinking water studies: Schoental and Head, 1957 as reported by IARC, 1976. 14 male and 6 female Wistar rats were administered riddelliine via drinking water (0.02 mg/mL) for 6 months. Five females and 5 surviving males were given 3 doses intraperitoneally (25 mg/kg body weight) in the 7^{th} month. The remaining 9 females continued to receive the alkaloid via drinking water. At one year, 4 males and 12 females received an additional i.p. dose of 30 mg/kg and were then observed until death. Liver nodules were observed in 4 males and 5 females. No nodules were observed in the livers of 8 male or 7 female controls.

9. Rat long-term drinking-water study: Hirono *et al*., 1977. Petasitenine extracted from flower stalks of wild *Petasites japonicus* Maxim (purity unknown) was given in drinking water to a group of 5 male and 6 female rats for up to 480 days. In the animals surviving longer than 5 months (4 males and 6 females), the incidence of hemangioendothelial sarcomas of the liver was 5/10 (1 male and 4 females), and the incidence of hepatocellular adenomas was 5/10 (3 males and 2 females). No liver tumors were seen in a group of 19 control rats (10 males and 9 females). When treated animals were compared to controls, the incidence of hemangioendothelial sarcomas was significantly increased in treated females ($p=0.01$) and in treated males and females combined ($p=0.002$).

10. Rat gavage study: Newberne and Rogers, 1973, as reported by IARC, 1976. A group of 50 male Sprague-Dawley rats were given weekly doses of monocrotaline (25 mg/kg) by gavage for 4 weeks, then 8 mg/kg for 38 weeks and sacrificed after 72 weeks. Of the animals surviving past week 46, 14/35 developed hepatocellular carcinomas.

11. Rat gavage study: Scheontal and Bensted, 1963, as reported by IARC, 1976. A single dose of retrorsine (30 mg/kg) by gavage to rats resulted in 5/29 hepatomas, although survival was poor and no controls were described.

12. Rat i.p. injection study: Svoboda and Reddy, 1972; as reported by IARC 1976. Twenty-five male Fischer 344 rats were given i.p. injections of 78 mg/kg-body weight lasciocarpine (10% of the LD50) twice weekly for 4 weeks and then once per week for 52 weeks. Seventy-six weeks after the first dose, 16 animals had developed tumors (10 hepatocellular carcinoma, 6 well differentiated squamous-cell carcinomas of the skin of the back, 5 pulmonary adenomas, and several gastrointestinal tumors). Among 25 control animals, 2 lung adenomas were observed.

13. Rat i.p. injection study: Schoental and Cavenagh, 1972, as reported by IARC, 1976. Three of 5 male rats given a single i.p. dose of hydroxysenkirkine (100-300 mg/kg-d) developed central nervous system (CNS) tumors. CNS tumors were reported to be rare in the colony of rats used.

14. Rat long-term i.p. injection study: Hirono *et al.* 1979a,b, as reported by IARC, 1983. This study examined the carcinogenicity of two of the alkaloids, senkirkine and symphytine. Groups of male ACI rats (20 per group, approximately 1 month of age) were administered i.p. injections at doses of 0 (vehicle control), 13 mg/kg body weight of symphytine, or 22 mg/kg body weight of senkirkine, twice weekly for 4 weeks, then once per week for 52 weeks. Animals were sacrificed after 650 days. No liver tumors were observed in the control group nor in 359 rats serving as controls for another experiment. In the symphytine-treated rats, 4/20 had liver tumors (3 hemangioendothelial sarcomas and 1 liver adenoma) ($p=0.05$, with simultaneous controls). In senkirkine-treated rats surviving greater than 290 days from the start of the injections, 9/20 developed hepatocellular adenomas ($p < 0.005$).

At least eight animal studies have been conducted to investigate the carcinogenic potential of pyrrolizidine alkaloid-containing plants or plant extracts. These are briefly described below.

Petasites japonicus

15. Rat long-term feeding study: Hirono *et al*., 1973. A group of 12 male and 15 female ACI rats (group 1) was fed diets supplemented with dried and milled young flower stalks of *P. Japonicus* at a concentration of 4% for six months and then given a diet supplemented with either 8% or 0% on alternate weeks for the duration of the experiment. Animals in group 2 (11 males and 8 females) received 4% *P. japonicus* in the diet throughout the experiment. A group of 7 males and 7 females served as controls. All surviving animals were killed and examined for tumors 480 days after the start of treatment. No tumors were found in the livers of the 14 control animals, but the incidence of hemangiosarcomas of the liver, hepatocellular adenomas and hepatocellular carcinomas were 3/27, 5/27 and 2/27, respectively, in group 1 and 9/19, 4/19 and 1/19 in group 2. The incidence of hemangiosarcomas in group 2 was significantly increased ($p=0.004$) above the incidence in controls.

16. Mouse long-term feeding studies: Fushimi *et al*., 1978. Groups of 20-24 male and 20-21 female Swiss mice, C57Bl/6 mice and ddN mice were fed diets supplemented with dried and milled young flower stalks of *P. japonicus* at a concentration of 4% for 480 days. No significant increase in tumor incidence at any site was found in treated Swiss or C57Bl/6 mice, but the incidence of lung adenomas or carcinomas (combined) in dosed ddN mice, 30/45, was significantly increased above the incidence of 1/50 in control ddN mice.

17. Hamster long-term feeding study: Fushimi *et al.*, 1978. A group of 13 male and 17 female Syrian golden hamsters was given feed supplemented with dried and milled young flower stalks of *P. japonicus* at a concentration of 4% for 480 days. No significant increase in tumor incidence at any site was found in dosed animals.

Senecio jacobaea

18. Rat drinking water study, Cook *et al.* 1950, as reported by IARC 1976. Eleven albino rats were administered mixed *Senecio jacobaea* L. alkaloids (of which seneciphylline is the major alkaloid) at concentrations of 0.1 or 0.05 mg/mL via drinking water intermittently over the first 11 months of life. The 3 rats treated for longer than 8 months developed tumor-like masses which were regarded as hepatomas. No controls were described.

19. Rat drinking water study, Schoental *et al.* 1954, as reported by IARC 1976. Thirteen male and 12 female Wistar rats were administered *S. Jacobaea* L. alkaloids in drinking water (0.05 mg/mL) for 1 or 2 weeks and water only for 7 weeks. 9 males and 1 female survived and were given a solution of 0.03 mg/mL of the alkaloids 3 times per week until death. "All developed nodular hyperplasia of the liver, some nodules being described as early trabecular hepatomas." These changes were not reported for the 7 male and 7 female controls.

20. Chicken i.v. injection study: Campbell 1956, as reported by IARC 1976. Twenty-four chickens were given weekly i.v. doses of 35, and later 20 mg/kg body weight of an alkaloid mixture from *S. Jacobaea* hydrochlorides (stated to mostly contain seneciphylline) for up to 8 weeks or until death. Liver tumors, 3 of which were malignant, developed in 6/18 birds that had died by the time of reporting (234 days). No information on controls was described by IARC.

Senecio longilobus

21. Rat long-term feeding studies; Harris and Chen, 1970, as reported by IARC 1976. This paper reported the results of 2 carcinogenicity experiments. In the first experiment, 295 Harlan rats (number of each sex not specified in IARC review) were administered dried *S. longilobus* in the diet at concentrations of 0.25% to 5.0%. Four males and 2 females treated for 133 to 446 days developed hepatomas, with one animal developing an additional tumor at 479 days. In the second experiment, Harlan rats were divided into groups of 50 animals of each sex. The first group was given a diet consisting of 0.75 % *S. longilobus*; all animals died within 131 days. The second group was fed a diet containing 0.5 % *S. longilobus*; however, only 4 rats survived longer than 200 days. The third group was administered a diet containing 0.5 % *S. longilobus* for 1 year (employing a "1 month on/2 weeks off" regimen). Of 23 rats surviving longer than 200 days, 3 males and 1 female developed heptocellular carcinomas. The fourth group was administered a diet containing 0.5 % *S. longilobus* for 1 year (employing a "1 week on/1 week off" regimen). Of the 47 rats surviving longer than 200 days, 14 males and 3 females developed malignant liver tumors (16 hepatocelluar carcinomas, 1 angiosarcoma). "Liver tumors were reported to be rare in 20 contemporary and many other non-contemporary controls."

Tussilago faraga

22. Rat long-term feeding study: Hirano *et al.* 1976, as reported by IARC 1976. Dried, milled flowers of coltsfoot, *Tussilago farfara* L. were administered to 3 groups of 1.5 month old ACI rats. In the first group, 6 females and 6 males received diets containing 32 % and 16 % of the herb, respectively. 8/12 rats developed hemangioendothelial sarcomas of the liver. Among these 8 tumor-bearing rats, one additional hepatocellular carcinoma, hepatocellular adenoma, and urinary bladder papilloma were reported. In the second group, 5 females and 5 males received diets containing 8% of the herb. A hemangioendothelial sarcoma of the liver developed in one of the 10 of the rats. In the third group, 5 females and 6 males received diets containing 4% of the herb. None of these rats developed tumors. None of these types of tumors were observed in contemporary control rats, 8 females and 9 males, nor in 150 controls in previous long-term experiments.

Comment on consistency of bioassay data: In the bioassays of the common putative toxic metabolites, the studies that administered dehydroretrenecine subcutaneously or dermally (#s 1-4) produced tumors primarily near the site of administration which is reasonable given the reactivity of the metabolite. The bioassay (#5) in which dehydroheliotridine was administered by i.p. injection increased the incidence of total tumors, which were distributed over multiple tissues, many of which were located in the abdominal cavity or on the intestines. This is consistent with the distribution of a reactive metabolite administered via the i.p. route. In studies of the parent alkaloids (#s 6-14) and in the studies of plant and plant-extracts (#s 15-22), the primary site of tumor development was the liver, which is reasonable considering that the liver is the primary site of metabolism to the reactive metabolites, dehydroretronecine and dehydroheliotridine.

Other relevant data

Dehydroretronecine and dehyroheliotridine alkylate numerous substrates including nucleic acids and amino acids, (especially cysteine and other sulfur-containing substrates) (Robertson, 1982; Peterson *et al.* 1983; Mattock and Bird, 1983). At physiological pH, the enantiomeric hydoxyl group of either metabolite is lost, forming a carbonium ion which alkylates DNA (Robertson 1982). Dehydroretronecine also cross-links DNA (Reed *et al.,* 1988). Positive genotoxic responses for dehydroretronecine were reported in the following test systems: *in vitro* transformation of a subclone of BHK21/C13 kidney cells (Styles *et al.*, 1980), sister chromatid exchange in human lymphocytes, and mutations in *Salmonella typhimurium* (TA92/base substitution) (Ord *et al.,* 1985). Dehydroretronecine and dehydroheliotridine are also potent antimitotic agents (Hsu *et al.,* 1973, IARC 1976, Mattocks and Legg, 1980).

Pyrrolizidine alkaloids are metabolized primarily in the liver to give hydrolysis products, N-oxides and dehydropyrrolizidine derivatives (IARC, 1976). The dehydropyrrolizidine derivatives are the most toxic and are formed by the mixed function oxidases in the liver. The initial metabolic product formed from alkaloids that are esters of retronecine (e.g., jacobine, monocrotaline, retrorsine, riddelliine, and seneciphylline) is believed to be dehydroretronecine (IARC 1976). Alkaloids that are esters of otonecine (e.g., hydroxysenkirkine and senkirkine) are also converted to dehydroretronecine, but must undergo a demethylation step to an intermediate which spontaneously rearranges to dehydroretronecine (IARC, 1976). Dehydroheliotridine is the major water soluble metabolite of lasiocarpine (IARC, 1976), and presumably other esters of heliotridine such as petasitenine, and symphytine (IARC, 1976; IARC, 1983). There is also some evidence that microsomes transform these pyrrolizidine alkaloids to a racemic mixture (+/-) rather than to pure enantiomers of dehydroretronecine (-) and dehyroheliotridine (+) (Kedzierski and Buhler, 1985).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over pyrrolizidine alkaloids which are metabolized to dehydroretronecine or its enantiomer dehyroheliotridine. Dehydroretronecine produced increased incidences of rhabdomyosarcomas in male rats in two injection studies and malignant skin tumors in female mice administered either by topical application or by subcutaneous injection. Dehyroheliotridine given to rats i.p. produced an increased incidence of total tumors. Parent compounds of the metabolites (including hydroxysenkirkine, lasiocarpine, monocrotaline, petasitenine, retrorsine, senkirkine, symphytine, or riddelliine) given to rats by oral or i.p. routes produced increase incidences of liver tumors and other cancers. Additionally, oral administration of pyrrolizidine alkaloid-containing plants or extracts to rats, mice or chickens induced liver tumors and cancers at other sites. The level of concern is increased because of positive findings of genotoxicity of dehydroretronecine in several test systems, as well as evidence of alkylating and antimitotic activity of both dehydroretronecine and dehyroheliotridine.

There is a **HIGH** level of **concern over the extent of exposure** because of the ingestion of pyrrolizidine alkaloid-containing plants or extracts commonly used in herbal remedies and teas. Potential exposures also come from consumption of pyrrolizidine alkaloid-containing honey or through the consumption of milk from livestock that consume pyrrolizidine alkaloid-containing plants.

References

American Herbal Products Association (AHPA 1997). *Botanical Safety Handbook*. McGuffin M, Hobbs C, Upton R, Godberg A (eds.). CRC Press, Boca Raton.

Allen JR, Hsu I-C, Carstens LA (1975). Dehydroretronecine-induced rhabdomyosarcomas in rats. *Cancer Res* **35**(4):997-1002.

Cook JW, Duffy E, Schoental R (1950). Primary liver tumours in rats following feeding with alkaloids of Senecio jacobaea. *Br J Cancer* **4**:405-410.

Coute CE, Hopley J, Hanley AB (1996). Metabolic activaiton of pyrrolizidine alkaloids by human, rat, and avacado microsomes. *Toxicon* **34**(9) 1058-1061.

Fushimi K, Kato K, Kato T, Matsubara M, Hirono I (1978). Carcinogenicity of flower stalks of *Petasites Japonicus* Maxim in mice and Syrian golden hamsters. *Toxicol Lett* **1**:291-294.

Hirono I, Shimizu M, Fushimi K, Mori H, Kato K (1973). Carcinogenic activity of *Petasites Japonicus* Maxim, a kind of coltsfoot. *Gann* **64**: 527-528.

Hirono, I, H Mori, K Yamada, Y Hirata, M Haga, H Tatematsu, S Kanie (1977). Brief Communication: Carcinogenic activity of petasitenine, a new pyrrolizidine alkaloid isolated from *Petasites japonicus* Maxim. *J Nat*

Cancer Inst **58**(4):1155-1157.

Hirano I, Haga M, Fujii M, Matsuura S, Matsubara N., Nakayama M, Furuya T, Kikichi M, Takanashi H, Uchida E, Hosaka S, Ueno I (1979a). Induction of hepatic tumors in rats by sindirkine and symphtine. *J Nat Cancer Inst* **63**: 469-472.

Hirono I, Mori H, Haga M, Fujii M, Yamada K, Hirata Y, Takanashi H, Uchida E, Hosaka S, Ueno, I, Matsushima T, Umezawa K, Shirai A (1979b). Edible plants containing carcinogenic pyrrolizidine alkaloids in Japan. In: Miller EC, Miller JA, Hirano I, Sugimura T, Takayama S (eds.) *Naturally Occurring Carcinogens-Mutagens and Modulators of Carcinogenesis*, Tokyo/Baltimore, Japan Scientific Society Press/University park Press, pp.79-87.

Hsu IC, Allen JR, Chesney CF (1973). Identification and toxicological effects of dehydroretronecine, a metabolite of monocrotaline. *Proc Soc Exp Biol* **144**:834-838.

International Agency for Research on Cancer (IARC 1976). General information and conclusions on pyrrolizidine alkaloids. *IARC monographs on the evaluation of the carcinogenic risks of chemicals to humans. Some naturally occurring substances*. Volume 10. pp. 333-342. IARC, Lyon, France.

International Agency for Research on Cancer (IARC 1983). Senkirkine. *IARC monographs on the evaluation of the carcinogenic risks of chemicals to humans. Some food additives, feed additives and naturally occurring substances*. Volume 31, pp. 231-238. IARC, Lyon, France.

International Agency for Research on Cancer (IARC 1987). General information and conclusions on pyrrolizidine alkaloids. *IARC monographs on the evaluation of the carcinogenic risks of chemicals to humans. Overall evaluations of Carcinogenicity : an updating of IARC monographs volumes 1-42.* Supplement 7. IARC, Lyon, France.

Johnson WD, Robertson KA, Pounds, JG, Allen JR (1978). Dehydroretronecine-induced skin tumors in mice. *J Nat Cancer Inst* **61**(1) 85-89.

Kedzierski B, Buhler DR (1985). Configuration of necine pyrroles – toxic metabolites of pyrrolizidine alkaloids. *Toxicol Lett* **25**(2):115-119.

Knowledge Access, Inc. (KAI 1995). Chapter 42. *Foodborne Pathogenic Microorganisms and Natural Toxins*. Knowledge Access, Inc.

Mattocks AR, Bird I (1983). Alkylation by dehydroretronecine, a cytotoxic metabolite of some pyrrolizidine alkaloids: an in vitro test. *Toxicol Lett* **16**:1-8.

Mattocks AR, Carbral (1982). Carcinogenicity of some pyrrolic pyrrolizidine alkaloid metabolites and analogues. *Cancer Lett* **17**:61-66.

Mattocks AR, Legg RF (1980). Antimitotic activity of dehydroretronecine, a pyrrolizidine alkaloid metabolite, and some analogous compounds, in a rat liver parenchymal cell line. *Chem Biol Interact* **30**(3):325-336.

Newberne PN, Rogers AE (1973). Nutrition, monocrotaline and aflatoxin B1 in liver carcinogenesis. In: Newman PN (ed.) *Plant Foods for Man*, pp. 23-31.

Ord MJ, Herbert A, Mattocks, AR (1985). The ability of bifunctional and monofunctional pyrrole compounds to induce sister-chromatid exchange (SCE) in human lymphocytes and mutations in Salmonella typhimurium. *Mutat Res* **149**:485-493.

Peterson JE, Jago MV, Reddy JK, Jarrett RG (1983). Neoplasia and chronic disease associated with the prolonged

administration of dehydroheliotridine to rats. *J Nat Cancer Inst* **70**(2): 381-386.

Reed RL, Ahern KG, Pearson GD, Buhler DR (1988). Crosslinking of DNA by dehydroretronecine, a metabolite of pyrrolizidine alkaloids. *Carcinogenesis* **9**(8):1355-1361.

Robertson KA (1982). Alkylation of N2 in deoxyguanosine by dehydroretronecine, a carcinogenic metabolite of the pyrrolizidine alkaloid monocrotaline. *Cancer Res* **42**:8-14.

Scheontal R, Bensted JPM (1963). Effects of whole body irradiation and of partial hepatectomy on the liver lesions induced in rats by a single dose of retrorsine, a pyrrolizidine (*Senecio*) alkaloid. *Br J Cancer* **17**:242-251.

Schoental R, Cavenagh JB (1972). Brain and spinal cord tumours in rats treated with pyrrolizidine alkaloids. *J Nat Cancer Inst* **49**:665-671.

Schoental R, Head MA (1957). Progression of liver lesions produced in rats by temporary treatment with pyrrolizidine (*Senecio*) alkaloids and the effects of betaine and high casein diet. *Br J Cancer* **11**:535-544.

Schoental R, Head MA, Peacock PR (1954). *Senecio* alkaloids: primary liver tumours in rats as a result of treatment with (I) a mixture of alkaloids from *S. jacobaea* Linn., (ii) retrorsine, (iii) isatidine. *Br J Cancer* **8**:458-465.

Shumaker RC, Robertson KA, Hsu IC, and Allen JR (1976). Neoplastic transformation in tissues of rats exposed to monocrotaline or dehydroretronecine. *J Nat Cancer Inst* **56**:787-790.

Styles J, Ashby J, Mattocks AR (1980). Evaluation in vitro of several pyrrolizidine alkaloid carcinogens: observations on the essential pyrrolic nucleus. *Carcinogenesis* **1**:161-164.

CARCINOGENICITY DATA SUMMARY: SESAMOL

Sesamol (3,4-(methylenedioxy)phenol; CAS No. 533-31-3), is a minor component (0.006-0.01%) of sesame oil. Bleached or hydrogenated sesame oil may contain as much as 0.1% sesamol. Estimated consumption of sesamol from sesame oil in the U.S. is at least 28,000 pounds per annum 1981-1986 (NIEHS, 1997). In addition, sesamol is also used in the pharmaceutical industry as a solvent and vehicle for fat soluble substances, and in the food industry as a powerful antioxidant to protect vegetable oils against rancidity. Sesamol is produced by acid hydrolysis of sesamolin, a glycoside in sesame seeds and oil (NIEHS, 1997). Sesamol does not appear to have GRAS status by the FDA.

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to sesamol were found in the literature.

Animal bioassays

1. Rat 104-week feeding studies: Hirose *et al.*, 1990. Groups of 30 male and 30 female F344 rats were given 0 or 2% sesamol in the diet for 104 weeks. A significant increase in the incidence of forestomach papillomas was observed in both sexes of rats. In male rats, tumor incidences were 0/30 in controls compared with 10/29 in the treated animals ($p<0.001$). In female rats, tumor incidences were 0/30 in the controls compared with 14/30 in the treated group ($p<0.001$). An increase in forestomach squamous cell carcinomas was also observed in both sexes. In males, tumor incidences were 0/30 in controls and 9/29 in treated rats ($p<0.001$). In females, tumor incidences were 0/30 in controls and 3/30 in treated rats. Forestomach hyperplasia was observed in 100% of both the male and female rats.

2. Rat long-term feeding studies: Ambrose *et al.*, 1958. Separate groups of rats (approximately 5/sex/dose/grade sesamol) were fed either pure or commercial-grade sesamol at doses of 0, 0.008, 0.016, 0.03, 0.06, 0.125, 0.25, 0.5 or 1.0% in the diet for 400 to 634 days. The results of this study are difficult to interpret because of poor reporting and the small number of animals per dose group. No statistically significant dose-related tumors appear to have been observed at any one site. However, the authors note that "a total of 20 proliferative lesions occurred in 134 rats fed sesamol. Sixteen of these lesion were benign, two were malignant and two were questionable." All proliferative lesions occurred in rats fed sesamol > 0.016%. No tumors were observed in the control rats.

3. Mouse 96-week feeding studies: Hirose *et al.*, 1990; Tamano *et al.*, 1992. Groups of 30 male and 30 female $B6C3F_1$ mice were given 0 or 2% sesamol via the diet for 96 weeks. A significant increase in forestomach squamous cell carcinoma developed in both sexes. For males, tumor incidences were 0/27 in controls and 11/29 in treated mice ($p<0.001$). In females, tumor incidences were 0/29 in controls and 5/30 in treated animals ($p<0.05$). Forestomach hyperplasia was observed in 100% of male mice and 93% of female mice.

Other relevant data

Sesamol was mutagenic in the mouse lymphoma assay with and without metabolic activation. It was not mutagenic in *Salmonella* strains TA98, TA100, TA1535, TA1537 or TA1538, with or without various metabolic activation systems (Cameron 1986, as reported by CCRIS, 1994).

Ito *et al.* (1993) demonstrated that administration of sesamol (2% in the diet) to F344 rats increased cellular proliferation (labeling index and hyperplasia) as well as ulceration of the stomach epithelium within 7 days of administration. Kagawa *et al.* (1993) fed rats sesamol (2%) and observed forestomach hyperplasia. Papillary hyperplasia regressed after cessation of dose, however, basal cell hyperplasia did not. Labeling indices, likewise, remained high after cessation of dose.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over sesamol because it produced an increased incidence of forestomach squamous cell carcinomas in feeding studies in rats and mice of both sexes. Forestomach papillomas were also observed in male and female rats fed sesamol. The level of concern is reinforced by positive findings of genotoxicity in some test systems. Some data suggest that the tumor responses might be due, at least in part, to a mechanism of irritancy. However, such a determination would involve a detailed analysis which was not performed for the "screening" purposes of this data summary.

There is a **HIGH** level of **concern over the extent of exposure** because sesamol is present in common food products.

References

Ambrose AM, Cox AJ Jr, DeEds F (1958). Toxicological studies on sesamol. *J Ag Food Chem* **6**:600-603.

Cameron TP (1986). Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute, as reported by CCRIS database.

CCRIS (Chemical Carcinogenesis Research Information System, 1994). Record 1386.

Hirose, M, Fukushima S, Shirai, T, Hasegawa, R, Kato T, Tanaka H, Asakawa E, Ito N (1990). Stomach carcinogenicity of caffeic acid, sesamol and catechol in rats and mice. *Japan J Cancer Res* **81**:207-212.

Ito N, Hirose M, Takahashi S (1993). Cell proliferation and forestomach carcinogenesis. *Environ Health Perpect* **101**(Suppl 5):107-110.

Kagawa M, Hakoi K, Yamamoto A, Futakuchi M, Hirose M (1993). Comparison of reversibility of rat forestomach lesions induced by genotoxic and non-genotoxic carcinogens. *Jpn J Cancer Res* **84**(11):1120-9.

National Institute of Environmental Health Sciences (NIEHS, 1997). National Toxicology Program Database. Chemical safety information for sesamol [6 pages]. Available from URL: http://www.ntp-db.niehs.nih.gov.

Tamano S, Hirose, M, Tanaka H, Asakawa E, Ogawa K, Ito N (1992). Forestomach neoplasm induction in F344/DuCrj rats and B6C3F1 mice exposed to sesamol. *Jpn J Cancer Res* **83**(12):1279-85.

CARCINOGENICITY DATA SUMMARY: STYRENE

Styrene (CAS No. 100-42-5) is one of the most widely used chemicals in the U.S. It is estimated that 4 million tons were produced in 1992 in the US (IARC, 1994). Styrene is used in the manufacture of polystyrene plastics, protective coatings, polyesters, synthetic rubber, copolymer resins with acrylonitrile and butadiene, and as a chemical intermediate. US EPA is currently updating its evaluation on the toxicity and carcinogenicity of styrene. IARC (1994) reviewed styrene and concluded that the evidence of carcinogenicity in humans was inadequate and that the evidence in experimental animals was limited; however, IARC classified styrene as a Group 2B carcinogen: possibly carcinogenic to humans. In making the overall evaluation, IARC (1994) took into account a variety of supporting evidence, including evidence that styrene is metabolized to styrene-7,8-oxide, which binds covalently to DNA and is genotoxic in various *in vitro* and *in vivo* assays, evidence that styrene induces dose-related chromosomal damage in human whole blood cultures at low doses, and evidence that DNA and protein adducts and chromosomal damage have been detected in humans occupationally exposed to styrene. Since IARC's review, two additional epidemiological studies (Macaluso *et al*., 1996; Delzell *et al*., 1996) have been published and two animal bioassays commissioned by the Styrene Information and Research Council (SIRC, 1996 and 1998) were completed.

Carcinogenicity Data available:

Epidemiological studies

The epidemiological data were reviewed by IARC in 1994 and are summarized below. Most of the data were obtained from workers occupationally exposed to styrene in one of the three following industries: manufacture of styrene-butadiene rubber, manufacture and polymerization of styrene, and manufacture of reinforced plastic. In general, workers in the reinforced plastic industry were exposed to higher levels of styrene than those in the other two industries. In addition, workers in the reinforced plastic industry were less likely to have concurrent exposure to other agents, such as benzene and 1,3-butadiene, that are known to be associated with lymphatic and hematopoietic cancers. Of the epidemiological studies published, the data collected from the reinforced plastic industry are believed to be the most useful in evaluating the carcinogenicity of styrene in humans.

Manufacture of styrene-butadiene rubber

A number of earlier mortality studies of the rubber industry showed that working in the industry may be associated with elevated risk for cancers at various sites. However, workers in this industry are potentially exposed to many other chemicals besides styrene. In one study (McMichael *et al*., 1976, as cited in IARC, 1994) in which exposure to styrene-butadiene was specified, an association (relative risk [RR]=6.2; 99.9% confidence interval [CI], 4.1-13) between lymphatic and hematopoietic malignancies and employment in a workplace that used styrene-butadiene was found.

Meinhardt *et al*. (1982) studied two groups of workers in the styrene-butadiene rubber industry. An elevated rate of lymphatic and hematopoietic cancers was found in one group of workers (standard mortality rate [SMR] of 212, 95% CI, 97-402). The SMR for leukemias was 278 (95% CI, 90-648). The authors did not find any excess mortality or cancer deaths in the other group. Matanoski *et al*. (1990) studied a cohort of male workers in the styrene-butadiene industry and found no significant excess of cancer at any site for the total cohort. However, they observed an excess of all lymphatic and hematopoietic neoplasms in a subgroup of black production workers (SMR=5.1, 95% CI, 1.9-11; as reported in IARC, 1994). Three of the six malignancies were leukemias (SMR=6.6; 95% CI, 1.4-19; as reported in IARC, 1994). A case-control study of 59 cases of lymphohematopoietic cancer was conducted (Santos-Burgoa *et al*., 1992) within the cohort studied by Matanoski *et al*. (1990). They reported that exposure to 1,3-butadiene was significantly associated with lymphatic and hematopoietic cancers, whereas exposure to styrene was not.

In a recent study, Macaluso *et al*. (1996) studied leukemia mortality among 16,610 subjects employed at 6 styrene-butadiene rubber manufacturing plants. Based on work histories, exposure levels for styrene, 1,3-butadiene, and benzene of the workers were estimated. The authors reported that there was no evidence to suggest that exposure to styrene was associated with increased incidence of leukemia. However, they did find that exposure to 1,3-butadiene was associated with a dose-related increase in the occurrence of leukemia. Delzell *et al*. (1996) studied a group of workers employed for at least a year in the styrene-butadiene rubber industry. They estimated that approximately

75% of the subjects were exposed to 1,3-butadiene and 83% were exposed to styrene. More leukemia deaths were observed than expected within the cohort (SMR=131; 95% CI, 97-174). Excess leukemia deaths were found primarily among workers with a long work history and a long follow-up period, as well as among subjects who had worked in areas with the potential for relatively high exposure to 1,3-butadiene or styrene.

Manufacture and polymerization of styrene

Ott *et al*. (1980, as referenced in IARC, 1994) studied mortality rates and tumor incidence rates among workers employed by the styrene manufacture and polymerization industry between 1937 and 1960. They found mortality and all neoplasms were lower than expected. Bond *et al*. (1992, as referenced in IARC, 1994) updated the study, adding a further 11 years of observations. They also found mortality from all causes and all neoplasms was lower than expected (overall SMR=76; 95% CI, 70-82; cancer SMR=81; 95% CI, 69-95), but cancers of the hematopoietic system were higher than expected (SMR=144; 95% CI, 95-208). Besides styrene, the workers were also potentially exposed to other chemicals such as benzene, acrylonitrile, 1,3-butadiene, ethylbenzene, dyes, and pigments. Hodgson and Jones (1985) studied a group of workers who had worked for at least a year in the production, polymerization, and processing of styrene between 1945 and 1978. No measurements of exposure were provided in the study, but many other chemicals were present in the working environment. The authors found a significant increase in lymphatic and hematopoietic cancer among the exposed workers (standardized incidence ratio=250; CI, 67-640; as reported in IARC, 1994).

Manufacture of reinforced plastic

Okun *et al*. (1985, as referenced in IARC, 1994) studied 5,021 workers who had worked in two reinforced plastic boat-building facilities between 1959 and 1978. No elevated mortality or cancer incidence rates were observed. Coggon *et al*. (1987, as referenced in IARC, 1994) studied a cohort of workers in the reinforced plastics industry in Britain and reported no increase in either mortality rates or hematopoietic cancer. However, they did report a slight increase in cancers of the respiratory system among workers exposed to styrene (SMR=126, 95% CI, 94-166). Kogevinas *et al*. (1994, as referenced in IARC, 1994) followed the same cohort and reported that by 1990 the excess of lung cancer was less marked, with a SMR of 106 (95% CI, 84-132). Kogevinas *et al*. also studied a larger cohort of workers in Europe and found no excess in mortality from all causes (SMR=96; 95% CI, 92-100) or from all neoplasms (SMR, 91; 95% CI, 83-98). The mortality rate in exposed workers for cancers of the lymphatic and hematopoietic tissues was not elevated (SMR=96; 95% CI, 71-127) and was not associated with length of employment. For two cohorts in Britain and Denmark, however, there were moderate increases in mortality from lymphatic and hematopoietic cancer (Britain: SMR=121; 95% CI, 25-355; Denmark: SMR=122; 95% CI, 78-181). Wong *et al*. (1994, as referenced in IARC, 1994) studied a cohort of workers employed by reinforced-plastics plants in the US between 1948 and 1977. They reported significantly increased rates of tumors at various sites: oesophagus (SMR=198; 95% CI, 105-322), respiratory system (SMR=141; 95% CI, 120-164), cervix uteri (SMR, 284; 95% CI, 136-521), and other female genital organs (SMR=202; 95% CI, 107-345). They did not observe any excess in lymphatic and hematopoietic cancers (SMR=82; 95% CI, 56-117). For workers in jobs associated with high styrene exposure, the SMR for lymphatic and hematopoietic cancers was 141, and for the highest cumulative exposure, the SMR was 134 (95% CI, 5-373). Kolstad *et al*. (1994, as referenced in IARC, 1994) studied a group of male Danish workers employed by industries that had ever produced reinforced plastics, and found elevated rates of leukemia and lymphomas among some groups of workers. In workers who were first employed more than 10 years before, the standard incidence rate (SIR) for leukemia was 157 (95% CI, 107-222; as reported in IARC, 1994); the excess was due to cases in workers who were employed for less than one year. For those employed in 1964-70, the SIR for leukemia more than 10 years after first short-term employment was 2.3 (95% CI, 1.4-3.6); however, for workers with more than one year employment, the corresponding SIR was 1.0 (95% CI, 0.52-1.7). For workers with less than 10 years since first employment, the only significant increase was for lymphomas (SIR=1.7; 95% CI, 1.0-2.5), with similar increases for short- and long-term employees.

Animal bioassays

1. Mouse long-term inhalation studies: SIRC, 1998. (Data not published in peer-reviewed literature). Groups of 70 male and 70 female CD-1 mice were exposed to styrene at 0, 20, 40, 80, or 160 ppm, 6 hours a day, 5 days a week. Ten mice of each sex and dose group were sacrificed following 52 and 78 weeks of exposure. Due to increased mortality in female control mice, terminal sacrifice of females took place during week 98. Male mice were sacrificed after 104 weeks of exposure. Body weight gain was reduced in male and female mice exposed

to 80 or 160 ppm. Increased incidences of pulmonary bronchiolar-alveolar adenomas were observed in females [6/50, 16/50 ($p<0.05$), 16/50 ($p<0.05$), 11/50, and 24/50 ($p<0.01$) for the control, 20, 40, 80, and 160 ppm groups, respectively] and in males [15/50, 21/50, 35/50 ($p<0.01$), 30/50 ($p<0.01$), and 33/50 ($p<0.01$)]. An increased incidence of pulmonary bronchiolar-alveolar carcinomas was observed in the high-dose females [0/50, 0/50, 2/50, 0/50, and 7/50 ($p<0.01$)]. The incidence of this tumor was not significantly increased in the males [4/50, 5/50, 3/50, 6/50, and 7/50].

2. Mouse long-term gavage studies: NCI, 1979a. Groups of 50 male and 50 female $B6C3F_1$ mice were treated with 150 or 300 mg/kg body weight styrene by gavage in corn oil on five days per week for 78 weeks, followed by an observation period of 13 weeks. Control groups of 20 male and female mice received corn oil only. Body weights of treated females were slightly reduced, and survival was slightly reduced in high-dose males (20/20, 46/50, 39/50 for control, low- and high-dose groups, respectively) and females (18/20, 40/50, 38/50). There was a significant positive association between dosage and the incidence of combined adenomas and carcinomas of the lung in male mice [0/20, 6/44 (13.6%), and 9/43 (20.9%)]. The incidence of these tumors in the high-dose group was also statistically significantly increased as compared to the controls ($p=0.024$). However, NCI noted that the incidence of these tumors among untreated historical controls at the laboratory was 32/271 (12%) ($p=0.08$ when the incidence of the high-dose group is compared with that of the historical controls). No statistically significant differences were observed between tumor incidence at any sites in the treated female mice and controls. NCI reported that there was suggestive evidence to indicate administration of styrene is associated with an increased incidence of a combination of adenomas and carcinomas of the lung in male $B6C3F_1$ mice. However, NCI concluded that this bioassay did not provide convincing evidence for the carcinogenicity of styrene in $B6C3F_1$ mice of either sex. In their review of the bioassay, IARC (1994) noted that there was a significant increasing trend in the incidence of hepatocellular adenoma in female mice: 0/20, 1/44, and 5/43.

3. Mouse long-term gavage studies: NCI, 1979b. Groups of 50 male and 50 female $B6C3F_1$ mice were treated with 203 or 406 mg/kg body weight styrene in a mixture (solution of 70% styrene and 30% ß-nitrostyrene) in corn oil by gavage, three days per week for 78 weeks. This was followed by an additional 14-week observation period. Control groups of 20 male and female mice received corn oil only. Body weights of high-dose female mice were slightly reduced. Survival among males was 18/20 (control), 43/50 (low-dose), and 33/50 (high-dose), and among the female was 17/20, 47/50, and 38/50. The combined incidence of adenoma and carcinoma of the lung in male mice was: 0/20 (control), 11/44 (low-dose; $p=0.016$), and 2/43 (high-dose) (IARC, 1994). However, the trend test and the high dose to control comparison were not statistically significant. No other tests for tumors at any site in either male or female mice were statistically significant. NCI concluded that there was no convincing evidence for the carcinogenicity of a solution of ß-nitrostyrene and styrene in $B6C3F_1$ mice of either sex.

4. Mouse single prenatal exposure and long-term gavage studies: Ponomarkov and Tomatis, 1978, as cited in IARC, 1994. A group of 29 pregnant O20 mice received a single dose of 1,350 mg/kg styrene in olive oil by gavage on day 17 of gestation. A control group of 9 pregnant mice received olive oil alone. Groups of 45 male and 39 female progeny from the dams that received styrene were administered 1,350 mg/kg styrene by gavage once a week from weaning until 16 weeks of age. Control groups of 20 males and 22 females with no prenatal exposure received olive oil alone. Administration of styrene was stopped at 16 weeks because of high mortality related to treatment. The experiment was terminated at 120 weeks. In the progeny that received weekly administration of styrene, the combined incidence of lung adenomas and carcinomas was significantly ($p<0.01$) increased over that in the vehicle controls: males, 8/19 controls versus 20/23 treated, and females, 14/21 controls versus 32/32 treated. There was no treatment-related difference in the incidence of tumors at other sites in the progeny (IARC, 1994).

5. Mouse single prenatal exposure and long-term gavage studies: Ponomarkov and Tomatis, 1978, as cited in IARC, 1994. A group of 15 pregnant C57B1 mice received a single administration of 300 mg/kg in olive oil by gavage on day 17 of gestation. A control group of 5 pregnant mice received olive oil only. Groups of 27 male and 27 female progeny from the dams that received styrene were administered 300 mg/kg styrene in olive oil by gavage once a week from weaning up to 120 weeks. Control groups of 12 male and 13 female mice received

olive oil alone. There were no treatment-related effects on body weight or survival. There were no treatment-related difference in the incidences of tumors at any site in the progeny (IARC, 1994).

6. Mouse intraperitoneal injection study: Brunnemann *et al*., 1992, as cited in IARC, 1994. A group of 25 female A/J mice received intraperitoneal injections of 20 µmol styrene in olive oil three times a week for a total of 20 injections. A vehicle control group of 25 mice received olive oil alone. The study was terminated 20 weeks after the last injection. There was no treatment-related increase in the incidence of lung tumors (3/25 versus 1/25 in controls).

7. Rat long-term inhalation studies: SIRC, 1996. (Data not published in peer-reviewed literature). Groups of 70 male and 70 female Sprague-Dawley rats were exposed to styrene at 0, 50, 200, 500, or 1000 ppm for 104 weeks, 6 hours a day, 5 days a week. Ten rats of each sex and dose group were sacrificed after 52 weeks of exposure. Body weight gain was reduced in groups exposed to 500 or 1000 ppm, particularly during the first year of the study. Survival by male rats was not affected by styrene exposure at levels up to 1000 ppm for 104 weeks. There was increased survival of females exposed to styrene at 500 or 1000 ppm. In rats killed at 52 weeks or later, there was a significant dose-related increase of interstitial cell tumors of the testes in male rats (2/60, 2/60, 2/60, 4/54, and 6/52 for the control, 50 ppm, 200 ppm, 500 ppm, and 1000 ppm groups, respectively). However, pairwise comparisons of the high-dose groups against the control group were not statistically significant. Comparison with historical control data showed that the incidences of interstitial cell tumors in the high-dose groups were within the background range.

8. Rat 52-week inhalation studies: Maltoni *et al*., 1982 and Conti *et al*. 1988, as referenced in IARC, 1994. Groups of 30 male and 30 female Sprague-Dawley rats were exposed by inhalation to 25, 50, 100, 200 or 300 ppm styrene for 4 hour per day, five days a week, for 52 weeks. The control groups were comprised of 60 male and 60 female rats. The study was terminated when the last animal died. No treatment-related effects on body weight or survival were reported. There was a significant ($p<0.01$, Cochran-Armitage trend test) correlation between dose and incidence of malignant mammary tumors in female rats: 6/60 controls, 6/30 at 25 ppm, 4/30 at 50 ppm, 9/30 at 100 ppm, 12/30 at 200 ppm and 9/30 at 300 ppm. The incidence rates of the three highest dose groups were also significantly higher than that of the controls ($p<0.05$, Fisher exact test for the 100, 200, and 300 ppm groups). The combined incidence of benign and malignant mammary tumors was also greater in treated female rats than in controls (34/60, 24/30, 21/30, 23/30, and 25/30).

9. Rat long-term drinking water studies: Beliles *et al*., 1985. Groups of 50 male and 70 female Sprague-Dawley rats were treated with 125 or 250 ppm styrene (nominal concentrations) daily in the drinking water for 104 weeks. Control groups of 76 male and 104 female rats received drinking water without styrene. At 52 weeks, 10 rats per sex and group were removed and killed. There was a significant reduction in water consumption among treated male and female rats and a significant reduction in body weight among high-dose females. There was no treatment-related effect on survival and no evidence of carcinogenicity. IARC (1994) reviewed the study results and noted the low level of exposure.

10. Rat long-term gavage studies: NCI, 1979a. Groups of 50 male and 50 female Fischer 344/N rats were treated with 1,000 or 2,000 mg/kg body weight styrene by gavage in corn oil five days per week for 78 weeks. Because of high mortality in the high-dose groups by week 23, additional groups of 50 male and 50 female rats administered 500 mg/kg styrene for 103 weeks were included in the study. Control groups of 20 male and 20 female rats received corn oil only. Treatment was followed by an observation period of 27 weeks for high- and mid-dose rats, and 1 week for low-dose rats. Survival was poor in both high-dose males and females. There was no treatment-related increase in the incidence of any type of tumor in male or female rats. NCI concluded that there was no convincing evidence for the carcinogenicity of styrene in Fischer 344 rats of either sex.

11. Rat long-term gavage studies: NCI, 1979b. Groups of 50 male and 50 female Fischer 344/N rats were treated with 350 and 700 mg/kg body weight (males) or 175 and 350 mg/kg bw (females) styrene in a mixture (solution of 70% styrene and 30% ß-nitrostyrene) in corn oil by gavage. The chemical was administered three days per week for 78 weeks, followed by an additional observation period of 29 weeks. Control groups of 20 male and 20 female rats received corn oil only. The body weights of male rats were slightly reduced. There was no effect

on survival in male (16/20, 34/50, 31/50) or female (12/20, 33/50, 31/50) rats (IARC, 1994). There was no treatment-related increase in the incidence of any type of tumor in male or female rats. NCI concluded that there was no convincing evidence for the carcinogenicity of a solution of β-nitrostyrene and styrene in Fischer 344/N rats of either sex.

12. Rat 52-week gavage studies: Conti *et al*., 1988, as referenced in IARC, 1994. Groups of 40 male and 40 female Sprague-Dawley rats were treated with 0, 50 or 250 mg/kg body weight styrene in olive oil by gavage on four to five days per week for 52 weeks. The study was terminated when the last animal died. There was no treatment-related effect on body weight; survival of female rats receiving the high dose was reduced. There was no treatment-related increase in the incidence of any type of tumor.

13. Rat single prenatal exposure and long-term gavage studies: Ponomarkov and Tomatis, 1978, as referenced in IARC, 1994. A group of 21 pregnant BDIV rats received a single administration of 1,350 mg/kg in olive oil by gavage on day 17 of gestation. A control group of 10 pregnant rats received olive oil only. Groups of 73 male and 71 female progeny of dams that received styrene were administered 500 mg/kg styrene in olive oil by gavage weekly from weaning up to 120 weeks. Control groups of 36 male and 39 female rats received olive oil alone. The experiment was terminated at 120 weeks. There were no treatment-related effects on body weight or survival. At the time of observation of the first tumor, 32 controls and 54 treated male progeny and 35 control and 68 treated female progeny were still alive. Stomach tumors occurred in three female rats (adenoma, fibrosarcoma, carcinosarcoma) administered styrene and in one female rat (fibrosarcoma) in the control group. There was no significant treatment-related increase in tumor incidence at any site (IARC, 1994).

14. Rat intraperitoneal injection studies: Conti *et al*., 1988 as referenced in IARC, 1994. Groups of 40 male and 40 female Sprague-Dawley rats were administered 4 intraperitoneal injections of 50 mg/animal in olive oil at two-month intervals. Control groups received injections of olive oil alone. The study was terminated when the last animal died. There was no treatment-related increase in the incidence of benign and/or malignant tumors.

Other Relevant Data:

Styrene is readily absorbed via inhalation and is mainly metabolized in the liver to styrene-7,8-oxide, a compound known to the State of California to cause cancer. Though most of the styrene-7,8-oxide produced in humans is detoxified by hydrolysis or conjugation with glutathione, styrene-7,8-oxide has been detected in blood samples of workers exposed to styrene in air (Korn *et al*., 1994; IARC, 1994). In *in vivo* and *in vitro* studies, it has been shown that styrene metabolites bind covalently to DNA, hemoglobin, and albumin (IARC, 1994). Using ^{32}P-post-labelling techniques, Horvath *et al*. (1994) found a significant linear relationship between styrene exposure and styrene-7,8-oxide-DNA adduct levels in the mononuclear cells of styrene workers. A dose-related increase of styrene-7,8-oxide-hemoglobin adduct levels was detected in a group of workers from a fiberglass reinforced plastic factory (Brenner *et al*., 1991 as referenced in IARC, 1994). Many cytogenetic studies had been conducted on workers occupationally exposed to styrene, with mixed results. Bonassi *et al*. (1996) performed a meta-analysis of 25 biomonitoring studies of occupational exposure to styrene. They found a significant increase of chromosomal aberrations was apparent in studies performed on workers with high levels of exposure to styrene, while inconclusive data were obtained for sister chromatid exchanges (SCEs) and micronuclei tests. Yager *et al*. (1993) found elevated levels of SCEs in blood lymphocytes of workers exposed to styrene. Recently, Yeowell-O'Connell *et al*. (1996) and Rappaport *et al*. (1996) reported that they detected styrene-7,8-oxide in the work room air of a plant that makes styrene reinforced plastics. It is possible that some of the cytogenetic effects observed in workers of the styrene reinforced plastic industry were related to direct exposure to styrene-7,8-oxide.

Most studies reported negative results for bacterial mutagenicity of styrene with or without exogenous metabolic activation (IARC, 1994). A few positive responses were reported in strains TA1535 and TA100 of *Salmonella typhimurium* in the presence of exogenous metabolic activation. Styrene was reported to be negative in inducing both forward gene mutation and mitotic gene conversion in the yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* in the presence of mouse liver microsomes (Barale, 1991). Cytogenetic studies on *Allium meristematic* root tips indicated that styrene was able to induce chromosome breaks, anaphase bridges and micronuclei. Styrene was shown to be weakly positive in inducing X-linked recessive lethal mutations in *Drosphila melanogaster* (Barale, 1991). Styrene has been shown to induce SCEs, chromosomal aberrations, and micronuclei in

human lymphocytes *in vitro* (IARC, 1994). Kligerman *et al*. (1993, as referenced in IARC, 1994) reported elevated levels of SCEs in rats and mice exposed to styrene via inhalation.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over styrene, since administration of the chemical has been shown to induce malignant lung tumors in female mice exposed via inhalation, and a dose-related increase in malignant and benign lung tumors in male mice exposed by gavage. In a study where pregnant mice and their offspring were exposed to styrene by gavage, elevated incidences of malignant and benign lung tumors were observed in the male and female offspring. In other inhalation studies, styrene produced a dose-related increase of testicular tumors in male rats, and malignant mammary tumors in female rats. The level of concern is supported by the human data suggesting a possible link between styrene exposure and hematopoietic cancer, as well as cancers of the respiratory system and female genital organs. Styrene has been shown to be clastogenic in numerous *in vitro* and *in vivo* tests in human and mammalian cells and mutagenic in some bacterial test systems. The level of concern is further reinforced by the detection of DNA damage, styrene-7,8-oxide-DNA adducts, and styrene-7,8-oxide-hemoglobin adducts in blood samples of workers occupationally exposed to styrene.

There is a **HIGH** level of **concern over the extent of exposure**. NIOSH (1983) estimated that 301,013 workers in 21,697 manufacturing facilities were potentially exposed to styrene at work. Occupational exposure to styrene is highest in the glass-reinforced plastic industry. Eight-hour time-weighted average air concentrations ranging from 20 to 100 ppm have been reported for this industry (IARC, 1994). Levels of 26 to 71 ppb styrene have been reported in indoor air in high-rise apartments (HSDB, 1995). The statewide mean ambient air concentration measured in California was 0.07 ppb in 1995; the highest sample measured contained 0.7 ppb styrene (ARB, 1998). Besides contaminated air, the general population is also exposed to styrene through the use of consumer products that contain styrene, by ingestion of food packaged in polystyrene, by ingestion of contaminated drinking water, and by inhalation of tobacco smoke (HSDB, 1995). Styrene has also been detected in human breast milk samples collected from four US cities (HSDB, 1995).

References

Air Resources Board (ARB, 1998). Air Resources Board Homepage. Statewide styrene summary data, 1990-1995. Available from http://arbis.arb.ca.gov/aqd/styr/ststate.htm.

Barale R (1991). The genetic toxicology of styrene and styrene oxide. *Mutat Res* **257**:107-126.

Beliles RP, Butala JH, Stack CR, Makris S (1985). Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. *Fund Appl Toxicol* **5**:855-868.

Bonassi S, Montanaro F, Ceppi M, Abbondandolo A (1996). Is human exposure to styrene a cause of cytogenetic damage? A re-analysis of the available evidence. *Biomarkers* **1**:217-225.

Delzell E, Sathiakumar N, Hovinga M, Macaluso M, Julian J, Larson R, Cole P, Muir DCF (1996). A follow-up study of synthetic rubber workers. *Toxicol* **113**:182-189.

Hodgson JT, Jones RF (1985). Mortality of styrene production, polymerization and processing workers at a site in Northwest England. *Scand J Work Environ Health* **11**:347-352.

Horvath E, Pongracz K, Rappaport S, Bodell WJ (1994). 32P-post-labelling detection of DNA adducts in mononuclear cells of workers occupationally exposed to styrene. *Carcinogenesis* **15**(7):1309-1315.

Hazardous Substances Data Bank (HSDB, 1995). National Library of Medicine. Bethesda, MD.

International Agency for Research on Cancer (IARC, 1994). *IARC monographs on the evaluation of carcinogenic*

risks to humans; Some industrial chemicals. Volume 60, pp. 233-320. IARC, Lyon, France.

Korn M, Gfrörer W, Filser JG, Kessler W (1994). Styrene-7,8-oxide in blood of workers exposed to styrene. *Arch Toxicol* **68**:524-527.

Macaluso M, Larson R, Delzell E, Sathiakumar N, Hovinga M, Julian J, Muir D, Cole P (1996). Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. *Toxicol* **113**:190-202.

Matanoski GM, Santos-Burgoa C, Schwartz L (1990). Mortality of a cohort of workers in the styrene-butadiene polymer manufacturing industry (1943-1982). *Environ Health Perspect* **86**:107-117.

Meinhardt TJ, Lemen RA, Crandall MS, Young RJ (1982). Environmental epidemiologic investigation of the styrene-butadiene rubber industry. Mortality patterns with discussion of the hematopoietic and lymphatic malignancies. *Scand J Work Environ Health* **8**:250-259.

National Cancer Institute (NCI, 1979a). Bioassay of styrene for possible carcinogenicity. National Cancer Institute, Carcinogenesis Technical Report No. 185. US Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI, 1979b). Bioassay of a solution of ß-nitrostyrene and styrene for possible carcinogenicity. National Cancer Institute, Carcinogenesis Technical Report No. 170. US Department of Health, Education, and Welfare, Public Health Service, National Institute of Health, Bethesda, MD.

National Institute for Occupational Safety and Health (NIOSH, 1983). Criteria for a recommended standard. Occupational exposure to styrene. NIOSH Publication No. 83-119, National Institute for Occupational Safety and Health, Cincinnati, OH.

Rappaport SM, Yeowell-O'Connell K, Bodell W, Yager JW, Symanski E (1996). An investigation of multiple biomarkers among workers exposed to styrene and styrene-7,8-oxide. *Cancer Res* **56**:5410-5416.

Santos-Burgoa C, Matanoski GM, Zeger S, Schwartz L (1992). Lymphohematopoietic cancer in sytrene-butadiene polymerization workers. *Am J Epidemiol* **136**:843-854.

Styrene Information and Research Council (SIRC, 1996). Styrene - 104-week repeat dose inhalation combined toxicity/carcinogenicity study in rats. Volume 1. Huntingdon Life Sciences.

Styrene Information and Research Council (SIRC, 1998). Styrene - 104-week repeat dose inhalation combined toxicity/carcinogenicity study in mice. Volume 1. Huntingdon Life Sciences.

Yager JW, Paradisin WM, Rappaport SM (1993). Sister-chromatid exchanges in lymphocytes are increased in relation to longitudinally measured occupational exposure to low concentrations of styrene. *Mutat Res* **319**:155-165.

Yeowell-O'Connell K, Jin Z, Rappaport SM (1996). Determination of albumin and hemoglobin adducts in workers exposed to styrene and styrene oxide. *Cancer Epidemiol Biomarkers Prevention* **5**:205-215.

CARCINOGENICITY DATA SUMMARY: TETRACHLORVINPHOS

Tetrachlorvinphos (2,4,5-trichloro-alpha-(chloromethylene) benzyl phosphate ester, 2-chloro-1-(2,4,5-trichlorophenyl)-vinyl dimethyl phosphate, Dietreen™, Gardona™, Rabon™, Stirohpos™; CAS No. 2489-77-2) is an organophosphorus insecticide used on livestock and food crops, and as a feed additive for beef and dairy cattle, horses, and swine. Approximately 45,000 kg (99,000 lbs) of tetrachlorvinphos were used in the U.S. in 1978 (IARC, 1983). Tolerances in the U.S. for residues of tetrachlorvinphos in or on raw agricultural commodities are 0.1-110 mg/kg; for a variety of 18 fodders the tolerance is 110 mg/kg, for fruits, grains, livestock and poultry fats, and eggs the tolerance is 0.1 mg/kg (IARC, 1983). IARC classified tetrachlorvinphos as a Group 3 carcinogen based on insufficient human data and limited evidence from animal studies (IARC, 1983). US EPA classified tetrachlorvinphos as a Group C carcinogen (US EPA, 1997). The compound was considered by the Proposition 65 Scientific Advisory Panel and rejected for listing on April 26, 1991. Since that time, additional data demonstrating positive genotoxicity of the compound have been published (Amer and Aly, 1992).

Carcinogenicity Data available:

Epidemiological studies

No human carcinogenicity studies were reported by IARC (1983) or found in a search of the more recent scientific literature by OEHHA.

Animal bioassays

1. Rat long-term dietary studies: NCI, 1978. Groups of 50 male and 50 female Osborne-Mendel rats were administered tetrachlorvinphos (technical grade, 98% purity) at one of two doses for 80 weeks, then observed for 31 additional weeks. Time-weighted average doses were either 4250 or 8500 ppm. Matched controls consisted of 10 untreated rats of each sex; statistical comparisons were based on both matched controls and pooled controls of 55 male and 55 female rats. The incidence of C-cell adenoma of the thyroid in female rats showed a significant dose-related trend (pooled controls, 1/46; low-dose, 2/50; high-dose, 7/46 ($p=0.013$)). The incidence of tumors found in the high-dose group was significantly higher than that of the pooled controls ($p=0.027$). The incidence of C-cell hyperplasia of the thyroid in both low-dose (7/50) and high-dose (16/46) females was significantly higher compared with that in pooled controls (1/50). In addition, cortical adenoma of the adrenal gland also showed a significant dose-related trend in the females (pooled controls, 0/50; low-dose, 2/49; high-dose, 5/50 ($p=0.017$)). The incidence of adenomas in the high-dose females was significantly higher than that of the pooled controls ($p=0.022$). Hemangioma of the spleen occurred in male rats at a significantly higher incidence in the low-dose group than in the pooled controls (controls, 0/52; low-dose, 4/48); however, neither the incidence in the high-dose group (0/47) nor the test for a dose-related trend was statistically significant. NCI concluded that administration of technical grade tetrachlorvinphos to Osborne-Mendel rats was associated with proliferative lesions of the C cells of the thyroid and cortical adenomas of the adrenal in females. IARC reviewed the bioassay and noted the short duration of treatment (80 weeks) and period of observation (31 weeks) prior to termination of the study (IARC, 1983).

2. Rat long-term dietary studies: Walker *et al*., 1972 as cited in IARC, 1983. Groups of 25 male and 25 female Porton rats were fed 5, 25, 125, or 2000 ppm (mg/kg) tetrachlorvinphos (95% pure, E-isomer) in the diet for 24 months. Groups of 45 males and 45 females served as controls. There were no significant differences in tumor incidence between treated and control rats. However, this study was limited by the relatively low doses administered and the small numbers of animals per dose group.

3. Mouse long-term dietary dietary studies: NCI, 1978. Groups of 50 male and 50 female $B6C3F_1$ mice were administered tetrachlorvinphos (technical grade, 98% purity) at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 12 additional weeks. Matched controls consisted of 10 untreated mice of each sex; statistical comparisons were based on both matched controls and pooled controls of 50 male and 50 female rats. The incidence of hepatocellular carcinoma in treated male mice was significantly higher than the controls (pooled controls, 5/49; low-dose, 36/50; high-dose, 40/50). The dose-related trend was also statistically significant ($p<0.001$). In females, the incidence of hepatocellular carcinoma was not significant (0/9, 2/48, 5/49, and 2/47 for the matched controls, pooled controls, low-dose, and high-dose groups, respectively); however, the

combined incidence of hepatocellular carcinomas and neoplastic nodules in the low- and high-dose groups (19/49 and 11/47, respectively) was significantly higher than that of the pooled controls (3/48). NCI concluded that in female mice the incidence of neoplastic nodules of the liver was associated with treatment, and in male mice tetrachlorvinphos was carcinogenic, causing hepatocellular carcinoma of the liver.

4. Mouse long-term dietary dietary studies: Parker *et al*., 1985. Groups of 80 male and 80 female $B6C3F_1$ mice were administered tetrachlorvinphos (98.7% purity) in the diet for two years at 17.5, 64, 320, 1600, 8000 or 16,000 ppm. Another group of 80 male and 80 female mice were fed 16,000 ppm tetrachlorvinphos of a different batch. There were 160 male and 160 female mice in the control groups. Ten treated and 20 control mice/sex/group were killed at 6, 12, and 18 months. Animals treated with 8000 and 16,000 ppm tetrachlorvinphos showed severely depressed weight gain, suggesting that the maximum tolerated dose might have been exceeded. Different results were reported by the two pathologists that examined the slides. According to the study pathologist, there was a significant ($p<0.05$) increase of hepatocellular carcinoma in one group of male mice exposed to 16,000 ppm (24/99 and 35/46 for controls and high-dose animals, respectively). However, according to the consultant pathologist, the incidences of hepatocellular carcinoma in these two groups were not statistically different (14/99 and 6/46 for controls and high-dose animals, respectively). The incidence of renal tubular adenoma and carcinoma was significantly increased ($p<0.05$) in male mice fed 16,000 ppm tetrachlorvinphos (1/99, 11/50 and 12/46 for controls, and the two high-dose groups, respectively). However, the two pathologists differed in their opinions on the malignancy of these tumors. The study pathologist considered the majority of these tumors to be carcinomas, while the consultant pathologist diagnosed most of the tumors as adenomas. The study pathologist also observed a significantly increased ($p<0.05$) incidence of hepatocellular carcinoma in one group of female mice exposed to 16,000 ppm tetrachlorvinphos (0/99 and 5/46 for controls and high-dose animals, respectively).

Other Relevant Data

Tetrachlorvinphos has been shown to induce structural chromosomal aberrations and sister-chromatid exchange in a primary culture of mouse spleen cells (Amer and Aly, 1992). It was shown to increase chromosomal aberration frequencies in Chinese hamster ovary cells without metabolic activation (Hazleton Laboratories**,** 1989 as cited in CDPR, 1996). Tetrachlorvinphos is not mutagenic in *Salmonella typhimurium* or *Escherichia coli*, either with or without metabolic activation (Shell, 1978 as cited in CDPR, 1996; Ruiz and Marzin, 1997; Bartsch *et al*., 1980 as cited in IARC, 1983). It did not increase mitotic gene conversion in stationary-phase cultures of *Saccharomyces cerevisiae* (Brooks *et al.*, 1982). Tetrachlorvinphos was reported to induce unscheduled DNA synthesis in human embryo fibroblasts (Benigni and Dogliotti, 1980 as cited in IARC, 1983). It forms DNA adducts in mouse liver following intraperitoneal injection (Zayed *et al*., 1983). Amer and Fahmy (1983) reported that tetrachlorvinphos induced micronuclei in mouse bone marrow after intraperitoneal and oral treatments.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over tetrachlorvinphos since it induced tumors at several sites in both sexes of mice and in female rats. Malignant liver tumors in male mice were caused by tetrachlorvinphos exposure in two separate studies. Malignant liver tumors were caused in one study, and combined malignant and benign liver tumors in another study in female mice. Also associated with tetrachlorvinphos exposure were kidney tumors in male mice in one study, and increased incidences of C cell adenomas of the thyroid and cortical adenomas of the adrenal gland in a study in female rats. The level of concern is reinforced by a number of genotoxicity tests showing tetrachlorvinphos can induce chromosomal damages *in vitro* and *in vivo*, and by the observation of DNA adduct formation in mouse liver.

There is **HIGH** level of **concern over the extent of exposure** since tetrachlorvinphos is a pesticide registered for use on fruits, vegetables, and livestock.

References

Amer SM, Aly FAE (1992). Cytogenetic effects of pesticides: IV. Cytogenetic effects of the insecticides Gardona and Dursban. *Mutat Res* 279:165-170.

Amer SM, Fahmy MA (1983). Cytogenetic effects of pesticides: II. Induction of micronuclei in mouse bone marrow by the insecticide gardona. *Mutat Res* 117:329-336.

Brooks TM, Dean BJ, Hutson DH, Potter D (1982). Microbial mutation studies with tetrachlorvinphos (gardona). *Mutat Res* 105:211-222.

California Department of Pesticide Regulation (CDPR, 1996). *Summary of toxicological data - tetrachlorvinphos*. April 4, 1986. Updated January 17, 1996.

International Agency for Research on Cancer (IARC, 1983). *IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans, miscellaneous pesticides*. Vol. 30. IARC, Lyon, France.

National Cancer Institute (NCI, 1978). *Bioassay of tetrachlorvinphos for possible carcinogenicity*. Technical report series no. 33., US Department of Health, Education, and Welfare, National Institutes of Health, DHEW publication no. (NIH) 78-833.

Parker CM, van Gelder GA, Chai EY, Gellatly JBM, Serota DG, Voelker RW, Vesselinovitch SD (1985). Oncogenic evaluation of tetrachlorvinphos in the B6C3F_1 mouse. *Fund Appl Toxicol* 5:840-854.

Ruiz MJ, Marzin D (1997). Genotoxicity of six pesticides by *Salmonella* mutagenicity test and SOS chromotest. *Mutat Res* 390:245-255.

US EPA (1997). *Health Effects Assessment Summary Tables. FY 1997 update*. Office of Research and Development, Office of Emergency and Remedial Response, United States Environmental Protection Agency, Washington, DC. EPA-540-R-97-036.

Zayed SMAD, Mostafa IY, Adam Y, Hegazi B (1983). *In vivo* methylation of guanine by the organophosphorus insecticide tetrachlorvinphos. *J Environ Sci Health* B18(6):767-779.

CARCINOGENICITY DATA SUMMARY: BLEOMYCIN AND ITS SALTS

Bleomycin (CAS No. 11056-06-7) is produced by *Streptomyces verticillus* bacteria. The sulfate or hydrochloride forms are used in human medicine as anti-neoplastic agents, either alone or in combination with other agents, in the treatment of squamous-cell carcinoma, Hodgkin's disease, non-Hodgkin's lymphoma and malignant neoplasms of the testes. Bleomycin was evaluated by IARC (1981, 1987) and classified in Group 2B, with inadequate evidence for carcinogenicity in humans and limited evidence for carcinogenicity in experimental animals.

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to bleomycin alone were found in an earlier search by IARC (1981, 1987) or more recently by OEHHA.

In a review of the literature, IARC (1981) reported that 15 patients with malignant lymphoma developed second malignant neoplasms following treatment with chemotherapy regimens containing bleomycin. Acute non-lymphocytic leukemia developed in 10 of the 15 patients, another developed a preleukemia syndrome and another a cutaneous malignant melanoma. All patients received multiple other cytotoxic agents and 12 received radiation therapy (IARC, 1981).

In a case-control study of treatment related secondary effects in testicular cancer patients (n=45) treated with a drug combination that included bleomycin and 22 controls, the frequency and stability of chromosomal aberrations was investigated (Gundy *et al*., 1990). Although the frequency of unstable chromosome aberrations decreased 36 months after termination of treatment, stable aberrations persisted up to 75 months post-treatment. No information was provided on the effects of individual drugs.

Animal bioassays

1. Rat 1-year subcutaneous (s.c) studies: Habs and Schmahl, 1984. Male and female Sprague-Dawley rats (30/sex/group) received weekly s.c. injections of bleomycin in saline for one year. The administered doses were 0, 0.35, 0.70, 1.4, or 2.8 mg/kg bw. The injection schedule for the two highest doses was changed to once/2 weeks after 10 weeks due to toxicity. Dose-dependent increased tumor incidences were observed for injection-site fibrosarcomas in males (0/30, 7/30, 6/30, 11/30 and 1/30; $p<0.05$ for pairwise comparison of the 3 lowest doses with controls by Fisher's exact test) and females (0/30, 7/30, 12/30, 9/30 and 2/30; $p<0.05$ for pairwise comparison of the 3 lowest doses with controls by Fisher's exact test) and for renal adenomas and adenocarcinomas (combined) in males (0/30, 1/30, 0/30, 7/30 and 3/30; $p<0.05$ for pairwise comparison of the second highest dose with controls by Fisher's exact test). The authors concluded that bleomycin caused injection-site and kidney tumors in rats.

2. Male rat 36-week intraperitoneal (i.p.) injection study: Shirai *et al*., 1984. Groups of 20 male F344 rats, initiated with 0.2% N-bis (2-hydroxypropyl)nitrosamine (DHPN) in the drinking water for 1 week, received a total of 34 weekly i.p. injections of 0 or 2 mg/kg bw bleomycin. An additional group of rats received bleomycin in the absence of DHPN. The experimental duration was 36 weeks. The lung adenocarcinoma tumor incidence was significantly increased ($p<0.05$) in the DHPN + bleomycin group, as compared with DHPN alone: DHPN-1/20, bleomycin-0/20, and DHPN+bleomycin-6/20. The authors concluded that bleomycin promoted the development of lung carcinomas in the DHPN-treated rats.

Other Relevant Data

Bleomycin is a clastogen, causing double and single strand breaks in DNA, most likely as a result of intercalation of a bleomycin-Fe^{2+} complex into DNA with the subsequent generation of reactive oxygen species (HSDB, 1996). In patients treated only with bleomycin, a significant increase in the number of chromosomal aberrations in bone marrow cells and peripheral lymphocytes has been observed (IARC, 1981).

In vitro, bleomycin increased chromosomal aberrations in human lymphocytes and lymphoblasts, a human cervical

carcinoma line (HeLa), mouse fibroblasts, Chinese hamster fibroblasts, Syrian hamster cell lines and in normal and abnormal human lymphoid cell lines. It induced sister chromatid exchanges in Chinese hamster fibroblasts. It is mutagenic in yeast and in *Drosophila melanogaster* and induced a dose-dependent increase in neoplastic transformation in a mouse cell line. No mutagenic activity has been observed in various Ames tests with or without S9 activation (IARC, 1981).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over bleomycin, which caused increased incidences of malignant and benign renal tumors in male rats, and injection-site fibrosarcomas in both sexes of the rat. There are also suggestions from case reports that bleomycin exposure may result in cancer in humans. The level of concern is increased by extensive supporting data. Bleomycin acts as a tumor promoter in a 36-week rat study. Chromosomal aberrations were increased in bone marrow cells and peripheral blood lymphocytes of individuals treated with bleomycin, and there were positive findings in multiple *in vitro* genotoxicity studies with human and mammalian cells.

There is a **MEDIUM** level of **concern over the extent of exposure** because bleomycin is used as an antineoplastic agent in the treatment of several types of human cancer. The potential for high doses of bleomycin to cause serious pulmonary toxicity lessens the likelihood that patients will receive high or long-term exposure, and reduces the level of concern. Occupational exposures to health care workers are of concern; NIOSH (1994) estimated that in 1983 8140 U.S. workers were occupationally exposed to bleomycin. Bleomycin is not produced in the U.S. (IARC, 1981).

References

Gundy S, Baki M, Bodrogi I, Czeizel A (1990). Persistence of chromosomal aberrations in blood lymphocytes of testicular cancer patients. I. The effect of vinblastine, cisplastin and bleomycin adjuvant therapy. *Oncol* **47**:410-414.

Habs M, Schmahl D (1984). Carcinogenicity of bleomycin sulfate and peplomycin sulfate after repeated subcutaneous applications to rats. *Oncol* **41**:114-19.

Hazardous Substances Data Bank (HSDB, 1996). National Library of Medicine. Bethesda, MD.

International Agency for Research on Cancer (IARC, 1981). Bleomycins (sulphates and hydrochlorides). *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: Some antineoplastic and immunosuppressive agents*. Volume 26, pp. 97-113. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of carcinogenic risks to humans. Overall evaluations of carcinogenicity: An updating of IARC monographs Volumes 1 to 42.* Supplement 7, p. 59. IARC, Lyon, France.

National Institute for Occupational Safety and Health (NIOSH, 1994). National Occupational Exposure Survey (1981-83), Cincinnati, OH.

Shirai T, Masuda A, Hirose M, Ikawa E, Ito N (1984). Enhancement of N-bis(2-hydroxypropyl)nitrosamine-initiated lung tumor development in rats by bleomycin and N-methyl-N-nitrosourethane. *Cancer Lett* **25**:25-31.

CARCINOGENICITY DATA SUMMARY: CHRYSOIDINE

Chrysoidine [4-(Phenylazo)-1,3-benzenediamine monohydrochloride, C.I. solvent orange 3; CAS No: 532-82-1] is used as a dye for silk and cotton; and as dye in oils, fats, and waxes for polishes, paper, leather, inks, wood and biological stains (IARC, 1975). It is also used to dye maggots used as fishing bait. IARC (1975) stated that according to U.S. industrial sources it was not used in food, drugs, or cosmetics. A derivative of chrysoidine, sulphamido-chrysoidine, is used in humans to treat and prevent streptococcal infection.

NIOSH (1983) estimated 5,039 workers in the U.S. were exposed, in 248 facilities. Annual production in the U.S. was estimated to be 20,000 lbs/year in 1981. Production and exposure data were not available for California. IARC (1975; 1987) reviewed chrysoidine and determined that there was limited evidence for carcinogenicity in experimental animals and inadequate evidence in humans; overall, IARC considered it to be not classifiable as to its carcinogenicity in humans. Studies (Sandhuy and Chipman, 1990; Sandhu and Chipman, 1991) reporting positive genotoxic activity of chrysoidine in human and rodent cells have been published since IARC's review.

Carcinogenicity Data available:

Epidemiological studies

Several case reports and case-control studies suggest an increased risk of bladder cancer in fishermen that used chrysoidine-dyed maggots as bait. Up to a 3-fold excess risk for using bronze maggots for >5 years was reported in one case-control study (IARC, 1987).

Animal bioassays

According to IARC (1975, 1987), only one set of bioassays is available which is adequate for evaluation of carcinogenicity. A very early non-positive study in rats (Maruya, 1938) was inadequately reported (IARC, 1975) and was not included in their evaluation.

1. Mouse long-term feeding studies: Albert, 1956. Groups of 60 each male and female mice were fed low vitamin diets containing 0.2% chrysoidine for 13 months, followed by control diet to the end of their lifespan. Two groups received a control dietary regime. In treated animals, an increased incidence of liver tumors was observed. The incidence of adenomas and adenocarcinomas (combined) was 75/104 (72%) (57% of 51 males and 87% of 53 surviving females), comprising 25 adenomas (24%) and 50 adenocarcinomas (48%). The first tumor was observed after 10-11 months. Metastases to the lung occurred in 3 females. Liver tumors, without metastases to the lungs, were observed in 1/89 (1%) and 2/117 (2%) in the two control groups. Leukemias and reticulum-cell sarcomas were observed in 28/104 (27%) treated animals and in 9/89 (10%) and 12/117 (10%) controls. The authors stated that liver neoplasms were promoted by a diet poor in vitamins (Albert, 1956). (This summary is based on the description in IARC (1975); the original report is in Polish and a translation was not available.)

Other relevant data

Chrysoidine is mutagenic in bacteria (*Salmonella* reverse mutation assay) with metabolic activation. It induced unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* and *in vivo* (Sandhu and Chipman, 1991, Sandhu and Chipman, 1990). UDS was also induced *in vitro* in human hepatocytes (Sandhu and Chipman, 1991). Chrysoidine has antifungal activity, inhibiting growth *in vitro*. Metabolism to the active mutagen is cytochrome P450 dependent (Sandhu and Chipman, 1990). Chrysoidine is an azo dye, a class of chemicals known to include human and animal carcinogens. In particular, it is chemically similar to the carcinogens *o*-amino-azotoluene and 2,4-diaminotoluene (Sandhu and Chipman, 1990).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over chrysoidine because it produced an increased incidence of liver adenomas and carcinomas in a feeding study in mice of both sexes. Carcinogenicity concern is increased

because of positive findings of genotoxicity in several test systems, by possible indications of carcinogenicity in humans, and by structural analogies with compounds known to be carcinogenic in animals and humans.

There is a **MEDIUM** level of **concern over the extent of exposure** because chrysoidine is present in a number of products (but not in food, drugs, or cosmetics). Some occupational exposures may result from production of the material and its incorporation into products, but no evidence was found to indicate its presence in the general environment. It is not clear whether chrysoidine-dyed maggots are used in California.

References

Albert Z (1956). Effect of prolonged feeding with chrysoidin on the formation of adenoma and cancer of the liver in mice. *Arch Immunol Ter Dosw* **4**:189-242

International Agency for Research on Cancer (IARC, 1975). *IARC monographs on the evaluation of carcinogenic risks to humans: Some aromatic azo compounds*. Volume 8, pp. 171-186. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of carcinogenic risks to humans. Overall evaluations of carcinogenicity: An updating of IARC monographs Volumes 1 to 42.* Supplement 7: p. 60. IARC, Lyon, France.

Maruya M (1938). On the renal changes of albino rats induced by the oral administration of 14 azo-compounds and 5 aromatic amino-compounds. *Tr Jap Path Soc* **28**:541-547.

National Institute for Occupational Safety and Health (NIOSH, 1983). National Occupational Hazard Survey.

Sandhu P, Chipman JK (1990). Bacterial mutagenesis and hepatocyte unscheduled DNA synthesis induced by chrysoidine azo-dye components. *Mutat Res* **240**:227-236

Sandhu P, Chipman JK (1991). Lack of release from hepatocytes *in vitro* or excretion *in vivo* of mutagenic metabolites. *Tox Letters* **58**:43-50.

CARCINOGENICITY DATA SUMMARY: N,N'-DIETHYLTHIOUREA

N,N'-diethylthiourea (CAS No. 105-55-5) is used as a corrosion inhibitor in metal pickling solutions and as an accelerator in the rubber industry.

Carcinogenicity Data available:

Epidemiological studies

No human carcinogenicity studies were found in the literature.

Animal bioassays

1. Rat long-term diet studies: NCI, 1979. Groups of 50 male and female Fischer 344 rats were given 125 and 250 ppm of N,N'-diethylthiourea in diet for 103 weeks. Twenty animals of both sexes were used as controls. There were increases in follicular-cell tumors of the thyroid in rats of both sexes, which were statistically significant in the high-dose groups. The combined incidences of follicular-cell adenomas and carcinomas in male rats were 0/18 in the control group, 1/45 in the low-dose group and 15/48 in the high-dose group (P = 0.004 by Fisher's exact test for high dose *vs*. control; dose-related trend P < 0.001 by Cochran-Armitage test). In female rats these combined incidences were control, 0/18; low dose, 4/46; and high dose 17/46 (P = 0.001 for high dose *vs*. control; dose-related trend P < 0.001). The incidences of follicular-cell carcinomas alone were control 0/18, low dose 1/45, high dose 11/48 in male rats and control 0/18, low dose 1/46, high dose 8/45 in female rats. This was statistically significantly elevated in the high-dose male rats (P = 0.021 for high dose *vs*. control), and the dose-related trend was significant in both sexes (males, P = 0.001; females P=0.006). NCI concluded that under the condition of the bioassay, N,N'-diethylthiourea was carcinogenic to Fischer 344 rats.

2. Rat 52-week diet study: Hasegawa *et al*., 1991. Two groups of 20 or 21 male Fischer 344 rats were given 0 or 200 ppm N,N'-diethylthiourea in diet for 52 weeks. At the end of the study, follicular cell carcinomas of the thyroid were observed in 1/21 rats treated with the chemical. C-cell adenomas of the thyroid were noted in two rats exposed to N,N'-diethylthiourea. None of these tumors were observed in the 20 control rats. The low incidence of such tumors in this study may be related to the less-than-lifetime duration of the study.

3. Mouse long-term diet studies: NCI, 1979. Groups of 50 male and female $B6C3F_1$ mice were given 250 and 500 ppm of N,N'-diethylthiourea in diet for 103 weeks. Twenty animals of both sexes were used as controls. Adequate number of animals in both groups survived sufficiently long to be at risk from late-developing tumors. Compound-related mean body weight depression was apparent among dosed male and female mice when compared to their respective controls, indicating that the concentrations used may have approximated the maximum tolerated dosages. No statistically significant positive association between administration of N,N'-diethylthiourea and increased tumor incidence was observed. NCI concluded that there was no evidence for the carcinogenicity of the compound in $B6C3F_1$ mice.

Other Relevant Data:

Structurally, N,N'-diethylthiourea is similar to thiourea, a group of chemicals that are known to have antithyroidal effects in both animals and humans. This effect may be related to the mechanism of carcinogenicity of these compounds. Two chemical in this group, ethylene thiourea and thiourea, are identified by the Proposition 65 program as carcinogens (OEHHA, 1998). N,N'-diethylthiourea has been shown to be positive in the mouse lymphoma cell forward mutation assay (McGregor *et al*., 1988).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern over** N,N'-diethylthiourea because it significantly increased in the incidences of malignant thyroid tumors in male rats, and of combined malignant and benign thyroid tumors in female rats. Additional concern comes from some evidence demonstrating N,N'-diethylthiourea's genotoxic potential, and from structure-activity analysis showing a relationship to other carcinogenic thiourea compounds.

There is a **MEDIUM** level of **concern over the extent of exposure**. NIOSH in its survey between 1981 and 1983 estimated that approximately 7,700 workers are potentially exposed to this compound in the USA. When released into the environment, N,N'-diethylthiourea is expected to be mobile in soil and present mostly in the aquatic phase in water. Volatilization from soil and water are not likely to occur to any significant extent. The estimated bioconcentration factor of 2 indicates that the chemical would not bioconcentrate in aquatic organisms in water. The half-life of N,N'-diethylthiourea in water is rather long as it was found to be stable towards hydrolysis and photolysis (HSDB, 1997).

References

Hasegawa R, Shirai T, Hakoi K, Wada S, Yamaguchi K, Takayama S (1991). Synergistic enhancement of thyroid tumor induction by 2,4-diaminoanisole sulfate, N,N'-diethylthiourea and 4,4'-thiodianiline in male F344 rats. *Carcinogenesis* **12**(8):1515-1518.

Hazardous Substances Data Bank (HSDB, 1997). National Library of Medicine. Bethesda, MD.

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C, Caspary WJ (1988). Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environ Mol Mutagen* **12**(1):85-154.

National Cancer Institute (NCI, 1979). Bioassay of N,N'-diethylthiourea for possible carcinogenicity. Technical report series no. 149. US Department of Health, Education, and Welfare, National Institutes of Health, DHEW publication no. (NIH) 79-1705.

Office of Environmental Health Hazard Assessment (OEHHA, 1998). Office of Environmental Health Hazard Assessment homepage: http://www.calepa.cahwnet.gov/oehha. Chemicals known to the State to cause cancer. Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

CARCINOGENICITY DATA SUMMARY: ISOPHOSPHAMIDE

Isophosphamide (holoxan, ifosfamide; CAS No. 3778-73-2) is related to the nitrogen mustards and is a synthetic analog of cyclophosphamide. It is used as an antineoplastic and immunosuppressive drug. Given intravenously, it is used in the treatment of oat-cell tumors of the lung, ovarian cancer, breast cancer, and non-Hodgkin's lymphomas (IARC, 1981). IARC (1981) reviewed isophosphamide and determined that there was limited evidence for carcinogenicity of the chemical in animal, and no data in humans. Since that review, additional evidence of genotoxicity has become available. IARC (1987) classified isophosphamide as a Group 3 carcinogen based on its 1981 review.

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Mouse 52-week intraperitoneal injection studies: NCI, 1977. Groups of 35 $B6C3F_1$ mice of each sex were given injections of either 10 or 20 mg/kg isophosphamide in buffered saline 3 times a week for 52 weeks. Fifteen mice of each sex were injected with vehicle alone and an additional 15 mice of each sex were used as untreated controls. Survival of the vehicle control males and treated males were poor. The incidence of hepatocellular adenomas and carcinomas was significantly elevated in the low-dose males ($p=0.043$); nevertheless, NCI concluded that these tumors were not clearly related to treatment. There was a dose-related increase of malignant lymphoma in female mice ($p=0.001$). The incidence of this tumor in high-dose females was significantly higher than that in either the vehicle controls ($p=0.005$) or the pooled controls ($p=0.001$). NCI concluded that isophosphamide was carcinogenic in female $B6C3F_1$ mice, producing malignant lymphomas of the hematopoietic system. The IARC (1981) Working Group considered comparisons with the small number of matched controls insufficient, and comparisons with the pooled controls suggestive, but inconclusive evidence of carcinogenicity.

2. Rat 52-week intraperitoneal injection studies: NCI 1977. Groups of 35 Sprague-Dawley rats of both sexes were given injections of 6 or 12 mg/kg isophosphamide in buffered saline 3 times a week for 52 weeks. Ten rats of each sex were injected with vehicle alone and an additional 10 rats of each sex were used as untreated controls. There was a dose-related increase of malignant lymphomas and leukemias in male rats using pooled vehicle controls ($p=0.032$) and a higher incidence in the high-dose males than in the pooled vehicle controls ($p=0.04$). However, these tumors were not statistically significant when compared with the matched vehicle control using adjusted analyses, and they cannot clearly be associated with treatment. NCI noted that 5 male rats with these tumors in the high-dose group had a median survival time of only 35 weeks. In female rats, there were significant increases of uterine leiomyosarcomas and mammary fibroadenomas in the low-dose group ($p<0.001$ for both tumors). The incidence of each tumor was also significantly increased when compared with matched vehicle controls using time-adjusted analyses. The incidence rates of uterine leiomyosarcoma and mammary fibroadenoma were lower in the high-dose group due to the low survival of this group. NCI concluded that isophosphamide was not carcinogenic in male Sprague-Dawley rats but was carcinogenic in female Sprague-Dawley rats. The IARC (1981) Working Group considered comparisons with the small number of matched controls insufficient, and comparisons with the pooled controls suggestive, but inconclusive evidence of carcinogenicity.

3. Mouse subcutaneous injection study: Mitrou *et al.*, 1979a. In a study of age-related susceptibility to treatment, 6 groups of female New Zealand Black/New Zealand White hybrid mice of 4 and 6 months old were given subcutaneous injections of 0.2 mg/day, 0.4 mg/day or 2 mg/week isophosphamide for 15 to 17 months. There were 23 to 34 animals in each of the four low- and medium-dose groups and 15 and 20 animals in the two high-dose groups (2 mg/week). Though the age, dosage and frequency of injection varied among the groups, a significant increase in the number of animals with tumors was observed in all exposed groups. The tumors

observed included 23 lymphomas, 7 undifferentiated sarcomas, 1 fibrosarcoma, 6 adenocarcinomas of the lung and 1 granulocytic leukemia (as reviewed by IARC, 1981).

4. Mouse subcutaneous injection study: Mitrou *et al.*, 1979b. The experiment described above was repeated with younger female mice of the same strain. Four groups of 9-10 mice, 6, 7, 8 or 12 weeks of age, were given subcutaneous injections of 0.2 mg isophosphamide 5 times per week. The experiment was terminated 7 or 8 months later. Only 1 treated animal died from a lymphoma; no additional tumors were detected among the exposed animals (as reviewed by IARC, 1981).

5. Mouse 8-week intraperitoneal injection studies: Stoner *et al.*, 1973. Groups of 10 A/He mice of each sex were given injections of either 18.8 or 47 mg/kg isophosphamide in water 3 times a week for 8 weeks. An additional group received 5 injections of 260 mg/kg each. Controls were 30 males and 30 females which received 24 injections of 0.1 ml water. Survivors were killed after 24 weeks. Significant increase in lung tumor incidences were observed in all treated groups (IARC, 1981).

Other Relevant Data

Isophosphamide was mutagenic in *Salmonella typhimurium* with metabolic activation. It also produced a dose-dependent increase in chromosomal aberrations in Chinese hamster bone-marrow cells following intraperitoneal injection (IARC, 1981). Wilmer *et al.* (1992) reported that when isophosphamide was administered to chicken embryos, the chemical induced a dose-related increase in sister chromatid exchange frequency in the B-cells. Isophosphamide is metabolized to isophosphoramide mustard, a member of the nitrogen mustards which are known to produce DNA damage and adducts (IARC, 1981).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** as isophosphamide has been shown to increase the incidence of uterine leiomyosarcoma and mammary fibroadenoma in female rats and malignant lymphoma in female mice. Administration of the chemical was also associated with increases of hepatocellular adenomas and carcinomas in male mice and lymphomas and leukemias in male rats, though the increases were not statistically significant. The concern is reinforced by the genotoxic activity of isophosphamide and the fact that it is metabolized to a nitrogen mustard.

There is a **MEDIUM** level of **concern over the extent of exposure** as the chemical is used as an antineoplastic and immunosuppressive drug. Patients using this drug are generally required to be under the close supervision of a physician experienced in the use of cancer chemotherapeutic agents. Though the general public is not likely to be inadvertently exposed to isophosphamide, occupational exposure to this chemical may occur.

References

International Agency for Research on Cancer (IARC, 1981). *IARC monographs on the evaluation of carcinogenic risk of chemicals to humans; Some antineoplastic and immunosuppressive agents*. Volume 26, pp. 237-247. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Overall evaluations of carcinogenicity: An updating of IARC monographs Volumes 1 to 42.* Supplement 7: p. 65. IARC, Lyon, France.

Mitrou PS, Fischer M, Mitrou G, Röttger P, Holtz G (1979a). The oncogenic effect of immunosuppressive (cytotoxic) agents in (NZBxNZW) mice. I. Long-term treatment with azathioprine and ifosfamide. *Arzneimittel-Forsch/Drug Res* **29**(1):483-488.

Mitrou PS, Fischer M, Mitrou G, Röttger P (1979b). The oncogenic effect of immunosuppressive (cytotoxic) agents in (NZBxNZW) mice. II. Emergence of tumors in young animals treated with azathioprine and ifosfamide, including a histologic assessment of the neoplasms. *Arzneimittel-Forsch/Drug Res* **29**(1):483-488.

National Cancer Institute (NCI, 1977). Bioassay of isophosphamide for possible carcinogenicity. US Department of Health, Education, and Welfare, Public Health Service, National Institute of Health. National Cancer Institute, Carcinogenesis Technical Report Series No. 32.

Stoner GD, Shimkin MB, Kniazeff AJ, Weisburger JH, Weisburger EK, Gori GB (1973). Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in Strain A mice. *Cancer Res* **33**:3069-3085.

Wilmer JL, Colvin OM, Bloom SE (1992). Cytogenetic mechanisms in the selective toxicity of cyclophosphamide analogs and metabolites towards avian embryonic B lymphocytes *in vivo*. *Mutat Res* **268**: 115-130.

CARCINOGENICITY DATA SUMMARY: 6-NITROBENZIMIDAZOLE

6-Nitrobenzimidazole (5-nitro-1H-benzimidazole; CAS No. 94-52-0) is used as an antifogging agent in photographic developers. According to NCI (1979), use as a photographic chemical is its sole commercial use; an experimental demonstration of its effectiveness as an antihelminthic does not appear to have been exploited. Details of production and exposure are scarce, but according to HSDB (1997), U.S. annual production was probably in excess of 1990 lbs/year (9.08 x 10^5 g/year) in 1975. NIOSH (1983) reported a total of 4272 employees in the U.S. potentially exposed. 6-Nitrobenzimidazole is listed on the EPA TSCA Chemical Inventory.

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to 6-nitrobenzimidazole were identified.

Animal bioassays

1. Mouse long-term feeding studies: NCI, 1979. 6-Nitrobenzimidazole was administered in the diet to groups of 50 $B6C3F_1$ male and 50 female mice. The diet fed to high-dose groups contained 0.24% 6-nitrobenzimidazole, while that fed to low-dose groups contained 0.12%. Concurrent control groups received plain diet. The study was initially set up with 0.06% as the low-dose and 0.12% as the high- dose, but it was decided on the basis of weight depression data that 0.12% was below the MTD and that 0.06% was too low to be useful. A new "high" dose group receiving 0.24% 6-nitrobenzimidazole in the diet, and a concurrent set of controls were started after 6 months; no histopathological examinations were performed on the original low-dose group. As a result of this change in study design, the two dosed groups had their own concurrent control group, but they were not mutually concurrent. Both sexes of mice showed statistically significant increased incidences of hepatocellular carcinomas in the high-dose groups. The incidence of hepatocellular adenoma was also significantly elevated in high-dose females. Liver tumor incidences were as follows: Adenoma in males - controls (result in control for low-dose group first): 0/50, 2/48; low-dose: 3/50; high-dose: 1/50. Carcinomas in males - controls: 12/50, 6/48; low-dose: 16/50; high-dose: 21/50 ($p < 0.01$). Adenoma in females - controls: 0/47, 0/50; low-dose: 2/44; high-dose: 9/47 ($p < 0.01$). Carcinoma in females - controls: 2/47, 1/50; low-dose: 2/44; high-dose: 11/47 ($p < 0.01$). (For significant results, p values are reported relative to the concurrent controls, by Fisher's Exact Test.)

2. Rat long-term feeding studies: NCI, 1979. 6-Nitrobenzimidazole was administered in the diet to groups of 50 Fischer 344 male and 50 female rats. The diet fed to high-dose groups contained 0.5% 6-nitrobenzimidazole, while that fed to the low-dose groups contained 0.12%. Control groups received plain diet. Similar dose-ranging problems to those noted in the mouse experiment were identified (the initial low-dose group of rats received 0.06% 6-nitrobenzimidazole in the diet), so the replacement 0.5% high-dose groups were started later with their own concurrent control groups. No significant dose-related increases in tumor incidence were observed. Non-neoplastic lesions which appeared to be dose-related were observed in the eye and Harderian gland. (The observation of non-neoplastic pathology in the Harderian gland, a known site in the rat for toxic and carcinogenic effects of chemicals metabolized to reactive intermediates, may suggest that this result is not necessarily an unequivocal "negative").

Other relevant data

6-Nitrobenzimidazole was positive in the *Salmonella* reverse mutation assay (Mortelmans, 1986).

Some other aromatic nitro-compounds are known to be carcinogenic, possibly via reduction to the corresponding amino compounds. Several imidazole compounds for which the liver appears to be a common target site are listed as known to cause cancer under Proposition 65.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over 6-nitrobenzimidazole because it produced an increased incidence of hepatocellular carcinomas in feeding studies in mice of both sexes. This concern is reinforced by the

observation of genotoxic effects, and by structural similarities to known carcinogens. The carcinogenicity concern is reduced because of the findings of carcinogenicity in the liver of mice only, with non-positive findings of carcinogenicity in rats.

There is a **MEDIUM** level of **concern over the extent of exposure**. 6-Nitrobenzimidazole is used as a photographic chemical, however, fairly small-scale commercial production occurs in the U.S. No data were found which allow evaluation of actual exposures from these activities, either in California or elsewhere, but significant exposure appears to be possible under some circumstances.

References

Hazardous Substances Data Bank (HSDB, 1997). National Library of Medicine. Bethesda, MD.

Mortelmans K (1986). *Salmonella* Mutagenicity tests. 2. Results from the testing of 270 chemicals. *Environ Mutagen* **8**(Suppl 7): 1-119.

National Cancer Institute (NCI, 1979). Bioassay of 6-Nitrobenzimidazole for Possible Carcinogenicity. Technical Report Series NCI-TR-117. US Department of Health, Education and Welfare, National Cancer Institute, Bethesda, MD.

National Institute for Occupational Safety and Health (NIOSH, 1983). National Occupational Hazard Survey.

CARCINOGENICITY DATA SUMMARY: PETASITENINE

Petasitenine (CAS No. 60102-37-6) is a naturally occurring pyrrolizidine alkaloid found in the herb *Petasites japonicus* Maxim. The young flower stalks of *P. japonicus* have long been used in Japan as a food and as an herbal remedy (cough remedy, expectorant). IARC (1983) evaluated evidence on the possible carcinogenicity of petasitenine and concluded that the evidence for carcinogenicity in laboratory animals was limited and there were no epidemiological studies or case reports available to the working group. IARC (1983)concluded that petasitenine is unclassifiable as to its carcinogenicity to humans (Group 3). An evaluation based on more recent versions of the inference guidelines used by IARC (1987) and other authorities might place greater emphasis on the supporting evidence. This includes the positive genotoxicity finding, and the structural similarities with compounds for which there is sufficient evidence of carcinogenicity in animals (see below).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to petasitenine were found in the literature.

Animal bioassays

1. Rat long-term drinking-water study: Hirono *et al*., 1977. Petasitenine extracted from flower stalks of wild *Petasites japonicus* Maxim (purity unknown) was given in drinking water to a group of 5 male and 6 female rats for up to 480 days. In the animals surviving longer than 5 months (4 males and 6 females), the incidence of hemangioendothelial sarcomas of the liver was 5/10 (1 male and 4 females), and the incidence of hepatocellular adenomas was 5/10 (3 males and 2 females). No liver tumors were seen in a group of 19 control rats (10 males and 9 females). When treated animals were compared to controls, the incidence of hemangioendothelial sarcomas was significantly increased in treated females (p=0.01) and in treated males and females combined (p=0.002).

2. Rat long-term feeding study: Hirono *et al*., 1973. A group of 12 male and 15 female ACI rats (group 1) was fed diets supplemented with dried and milled young flower stalks of *P. Japonicus* at a concentration of 4% for six months and then given a diet supplemented with either 8% or 0% on alternate weeks for the duration of the experiment. Animals in group 2 (11 males and 8 females) received 4% *P. japonicus* in the diet throughout the experiment. A group of 7 males and 7 females served as controls. All surviving animals were killed and examined for tumors 480 days after the start of treatment. No tumors were found in the livers of the 14 control animals, but the incidence of hemangiosarcomas of the liver, hepatocellular adenomas and hepatocellular carcinomas were 3/27, 5/27 and 2/27, respectively, in group 1 and 9/19, 4/19 and 1/19 in group 2. The incidence of hemangiosarcomas in group 2 was significantly increased (p=0.004) above the incidence in controls.

3. Mouse long-term feeding studies: Fushimi *et al*., 1978. Groups of 20-24 male and 20-21 female Swiss mice, C57Bl/6 mice and ddN mice were fed diets supplemented with dried and milled young flower stalks of *P. japonicus* at a concentration of 4% for 480 days. No significant increase in tumor incidence at any site was found in treated Swiss or C57Bl/6 mice, but the incidence of lung adenomas or carcinomas (combined) in dosed ddN mice, 30/45, was increased above the incidence of 1/50 in control ddN mice.

4. Hamster long-term feeding study: Fushimi *et al.*, 1978. A group of 13 male and 17 female Syrian golden hamsters was given feed supplemented with dried and milled young flower stalks of *P. japonicus* at a concentration of 4% for 480 days. No significant increase in tumor incidence at any site was found in dosed animals.

Other relevant data

Petasitenine was mutagenic in bacteria and in mammalian cells *in vitro*. It also induced chromosomal aberrations, unscheduled DNA synthesis and transformation in mammalian cells *in vitro*. The purity of the petasitenine samples used in these tests was not stated. IARC (1983) concluded that there was sufficient evidence that petasitenine is

active in short-term tests.

Petasitenine shows structural and metabolic similarities with some other pyrrolizidine alkaloids for which there is sufficient evidence of carcinogenicity in animals (see the data summary on pyrrolizidine alkaloids and their metabolites).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over petasitenine based on a limited study in rats in which hemangiosarcomas were associated with consumption of drinking water containing an extract of petasitenine of unknown purity and because the same type of tumors were observed in rats administered feed containing the natural plant source of petasitenine, *Petasites japonicus.* Consumption of *Petasites japonicus* by rats was also associated with an increased incidence of benign and malignant hepatocellular tumors, while consumption by mice was associated with benign and malignant lung tumors. The level of concern is raised by positive evidence from multiple test systems that petasitenine is genotoxic.

There is a **MEDIUM** level of **concern over the extent of exposure** to petasitenine because importation and consumption of traditional Asian herbal medications does occur in California, however, no information on the amounts imported or consumed are available.

References

Fushimi K, Kato K, Kato T, Matsubara M, Hirono I (1978). Carcinogenicity of flower stalks of *Petasites Japonicus* Maxim in mice and Syrian golden hamsters. *Tox Letters* **1**:291-294.

International Agency for Research on Cancer (IARC, 1983). *IARC Monographs on the Evaluation of Carcinogenic Risks of Chemicals to Humans.* Volume 31: Some food Additives, Feed Additives and Naturally Occurring Substances. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*. Supplement 7, IARC, Lyon, France.

Hirono, I, H Mori, K Yamada, Y Hirata, M Haga, H Tatematsu, S Kanie (1977). Brief Communication: Carcinogenic activity of petasitenine, a new pyrrolizidine alkaloid isolated from *Petasites japonicus* Maxim. *J Natl Cancer Inst* **58**(4):1155-1157.

Hirono I, Shimizu M, Fushimi K, Mori H, Kato K (1973). Carcinogenic activity of *Petasites Japonicus* Maxim, a kind of coltsfoot. *Gann* **64**: 527-528.

CARCINOGENICITY DATA SUMMARY: 1-BUTYLHYDRAZINE HYDROCHLORIDE

1-Butylhydrazine hydrochloride (CAS No. 56795-65-4) is a rocket fuel (liquid propellant) and industrial chemical.

Carcinogenicity Data available:

Epidemiological studies

No human carcinogenicity studies were found in the literature.

Animal bioassays

1. Mouse lifetime drinking water studies: Nagel *et al.,* 1975. Swiss albino mice (50/sex) were administered a 0.0125% solution of 1-butylhydrazine hydrochloride in the drinking water for life. Doses administered were equivalent to 1.3 mg/d for the females and 2.1 mg/d for the males. Female mice exhibited an incidence of 34/50 (68%, $p<0.001$) lung tumors (adenoma or adenocarcinoma). Treatment with 1-butylhydrazine hydrochloride significantly shortened the length of survival compared with control animals. The incidence of lung tumors in male mice was 19/49 (39%, $p<0.05$). Multiple lung tumors were observed in both male and female tumor-bearing mice. Among untreated male control mice 21/99 (21%) developed lung tumors. Female control mice demonstrated a lung tumor incidence of 23/99 (23%).

Other relevant data

Structurally-similar alkylhydrazines caused tumors at multiple sites. Nagel *et al.* (1975) (see above) also tested propylhydrazine hydrochloride at concentrations of 0.25% in drinking water. Lung tumor incidence for propylhydrazine-treated mice was equivalent to that observed for 1-butylhydrazine-treated mice. Toth *et al.* (1981) administered 1,1-di-*n*-butylhydrazine in drinking water to mice for life and observed increased incidences of tumors of the lung, forestomach and liver. Toth (1977) reviewed the evidence indicating that five substituted hydrazines induced cancers of the large bowel and other sites. These hydrazines included 1,1- and 1,2-dimethylhydrazine, methylhydrazine (which are listed under Proposition 65 as chemicals known to cause cancer), 1-methyl-2-butylhydrazine dihydrochloride and trimethylhydrazine hydrochloride. No genotoxicity studies on 1-butylhydrazine hydrochloride were found in the literature.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** for 1-butylhydrazine hydrochloride because of increased incidences of lung tumors in male and female mice, as well as strong evidence that structurally-similar alkylhydrazines also cause lung tumors and cancers at other sites.

There is a **LOW** level of **concern over the extent of exposure** because the extent of production and use of 1-butylhydrazine is not known to OEHHA at this time; however, there is the potential for contamination of drinking water with 1-butylhydrazine hydrochloride, as has occurred with other rocket propellants.

References

Nagel DH, Shimizu DH, Toth B (1975). Tumor induction studies with N-butyl- and N-propylhydrazine hydrochlorides in Swiss mice. *Eur J Cancer* **11**:473-478.

Toth B (1977). The large bowel carcinogenic effects of hydrazines and related compounds occurring in nature and in the environment. *Cancer* **40**(5 Suppl):2427-2431.

Toth B, Nagel D, Patil K (1981). Carcinogenic effects of 1,1-di-n-butylhydrazine in mice. *Carcinogenesis* **2**(7):651-654.

CARCINOGENICITY DATA SUMMARY: 3, 3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE

3,3'-Dimethoxybenzidine-4-4'-diisocyanate (CAS No. 91-93-0) has uses in isocyanate-based adhesive systems as a polyurethane elastomer component. It has been used as an experimental chemical for potential uses in coatings, gaskets, and shock absorbers (IARC, 1985; HSDB, 1995). Exposure is thought to be limited to researchers involved in experimental synthesis of elastomers and polymeric coatings (NCI, 1979). This agent is not produced commercially in the U.S. (IARC, 1986). IARC (1986; 1987) classified this agent as Group 3 based on no data in humans and limited evidence of carcinogenicity in animals. An evaluation based on more recent versions of the inference guidelines used by IARC and other authorities might place greater emphasis on the observation of tumors at multiple sites, the positive genotoxicity finding, and the structural similarities with (and hydrolysis to) a compound for which there is sufficient evidence of carcinogenicity in animals (see below).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to 3,3'-dimethoxybenzidine-4-4'-diisocyanate were identified in an earlier review by IARC (1986) or in a more recent literature search by OEHHA.

Animal bioassays

1. Rat long-term feeding studies: NCI, 1979. Groups of F344/N rats (50/sex/dose, with the exception of 49 high-dose females) were given 1500 or 3000 mg 3,3'-dimethoxybenzidine-4-4'-diisocyanate/kg body weight by gavage for the first 22 weeks of the study, and then subsequently in feed at concentrations of 22,000 and 44,000 ppm. The animals were administered the compound for 78 weeks, and then observed for 26 weeks. Twenty animals per group served as controls. A significant association between the incidence of leukemia and malignant lymphoma and dose of 3,3'-dimethoxybenzidine-4-4'-diisocyanate was observed among male and female rats, with statistically significant increases in incidence over controls among the low- and high-dose male rats, and high-dose female rats. Female rats showed an association between increased incidence of endometrial stromal polyps and dose. Male rats in both dose groups showed increased incidence of skin tumors (ear skin excepted). Squamous-cell carcinomas and sebaceous adenocarcinomas (combined) of the Zymbal gland and skin of the ear showed a significant increased trend with dose in both male and female rats. The purity of the administered compound was not stated. NCI concluded that the compound was carcinogenic to F344 rats of both sexes.

2. Mouse long-term feeding studies: NCI, 1979. Groups of $B6C3F_1$ mice (50/sex/dose) were given 1500 or 3000 mg 3,3'-dimethoxybenzidine-4-4'-diisocyanate/kg body weight by gavage for the first 22 weeks of the study, and then subsequently in feed at concentrations of 22,000 and 44,000 ppm 3,3'-dimethoxybenzidine-4-4'-diisocyanate. The animals were administered the compound for 78 weeks, and then observed for 25 weeks. Twenty animals per group served as controls. No statistically significant increase in tumor incidence was observed. The purity of the administered compound was not stated. NCI concluded there was no evidence of carcinogenicity in $B6C3F_1$ mice.

Other relevant data

3,3'-Dimethoxybenzidine-4-4'-diisocyanate has been shown to be mutagenic in the *S. typhimurium* mutagenesis assay with metabolic activation, but not without it, in strain TA98 (Haworth *et al.*, 1983; IARC, 1985). Results in *Salmonella* strains TA1535, TA1537, and TA100 were negative. 3,3'-Dimethoxybenzidine-4-4'-diisocyanate bears structural resemblance to 3,3'-dimethoxybenzidine and 3,3'-dimethoxybenzidine dihydrochloride, two compounds which have been shown to produce tumors in Fischer rats (Hadidian *et al.*, 1968; sufficient evidence of carcinogenicity in experimental animals according to IARC, 1982; and listed under Proposition 65 as known to cause cancer). 3,3'-Dimethoxybenzidine is the immediate hydrolysis product of 3-3'-dimethoxybenzidine-4,4'-diisocyanate.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over 3,3'-dimethoxybenzidine-4,4'-diisocyanate because of positive bioassay results showing the development of leukemia and malignant lymphoma and Zymbal gland tumors in male and female rats, and skin tumors in male rats. The compound has been shown to be mutagenic with metabolic activation in a single strain in the *Salmonella* mutagenesis assay, and upon hydrolysis formed the carcinogen, 3,3'-dimethoxybenzidine.

There is **LOW** level of **concern over the extent of exposure** because this compound is used in a limited experimental capacity and is not produced commercially. NIOSH in its 1983 National Exposure Survey estimated that 2 facilities and 227 employees in the U.S. were potentially exposed (RTECS, 1997).

References

Hadidian Z, Frederickson TN, Weisburger EK, Weisburger JH, Glass RM, Mantel N (1968). Tests for chemical carcinogens. Report on the activity of derivatives of aromatic amines, nitrosamines, quinolines, nitroalkanes, amides, epoxides, aziridines, and purine antimetabolites. *J Nat Cancer Inst* 41(4):985-1025.

Haworth S, Lawlor, T, Mortelmans K, Speck W, Zeiger E (1983). *Salmonella* mutagenicity test results for 250 chemicals. *Environ Mutagen Suppl.* **1**:3-142.

Hazardous Substances Data Bank (HSDB, 1995). National Library of Medicine. Bethesda, MD.

International Agency for Research on Cancer (IARC, 1982). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Industrial Processes and Industries Associated with Cancer in Humans, IARC Monographs Volumes 1 to 29*, Supplement 4, pp.116-8. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1985). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Some Chemicals Used in Plastics and Elastomers*, Volume 39, pp. 279-. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*. Supplement 7, p. 62. IARC, Lyon, France.

National Cancer Institute (NCI, 1979). Bioassay of 3,3'-Dimethoxybenzidine-4-4'-diisocyanate for Possible Carcinogenicity, CAS No. 91-93-0, NCI-CG-TR-128, US Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health.

Registry of Toxic Effects of Chemical Substances (RTECS, 1997). Database produced by the U.S. National Institute for Occupational Safety and Health.

CARCINOGENICITY DATA SUMMARY: ESTRADIOL MUSTARD

Estradiol mustard (CAS No. 22966-79-6) is used in clinical studies as an antineoplastic agent. The chemical has been proposed for use in patients with cancer of the ovary, breast, and prostate but there is no indication that the chemical is currently used to treat cancer. It is the ester of the estrogen, estradiol, and a nitrogen mustard alkylating agent, chlorphenacyl. IARC (1975; 1987) classified estradiol mustard as a Group 3 carcinogen based on limited evidence in animals and no data in humans. This classification was based on IARC's 1975 review, and did not include any review of the NCI (1978) bioassays.

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Mouse 52-week gavage studies: NCI, 1978. Groups of 34-36 B6C3F_1 mice of each sex were administered estradiol mustard at 15 or 30 mg/kg, three times per week for 52 weeks. Controls consisted of groups of 15 mice of each sex that were not administered the chemical and also groups of 14 male mice and 16 female mice administered the vehicle alone. All surviving mice were killed at 82-86 weeks. Statistically significant dose-related increases of lymphomas were observed in both male and female mice. Statistically significant increased rates of lymphoma or lymphocytic leukemia were observed in the low- and high-dose groups of both sexes. Alveolar/bronchiolar adenoma or carcinoma rates were significantly elevated in low-dose males and females compared with controls. Similarly, sarcoma of the myocardium occurred at an increased rate in low-dose males ($p=0.015$) and females ($p=0.002$) compared with the pooled vehicle controls. The survival of both male and female mice in the high-dose groups was lower than that of the respective low-dose groups and may account for the lower tumor incidence rates in the high-dose groups. Sarcoma of the myocardium is a rare tumor in this strain of mice; it was not observed in any of the more than 500 male and 500 female historical control mice. Squamous-cell carcinoma of the stomach occurred in treated male (high-dose 2/29) and female mice (low-dose 2/26, high-dose 2/14) but was absent in all controls. This is also a rare tumor as it was not observed in any of the more than 500 male and 500 female historical control mice of this strain. NCI concluded that estradiol mustard was carcinogenic in both male and female B6C3F_1 mice.

2. Rat 52-week gavage studies: NCI, 1978. Groups of 35 Sprague-Dawley rats of each sex were administered estradiol mustard at 0.62 or 1.25 mg/kg, three times per week for 52 weeks. Controls consisted of groups of 10 rats of each sex that were not administered the chemical and also groups of 10 rats of each sex administered the vehicle alone. All surviving rats were killed at 84-86 weeks. No statistically significant increase in tumors was observed in either male or female rats. However, there was a dose-related increase in the incidence of fibroadenoma of the mammary gland in male rats (pooled vehicle controls: 0/18; low-dose: 2/35; high-dose: 5/33). NCI concluded that estradiol mustard was not carcinogenic in Sprague-Dawley rats.

3. Mouse intraperitoneal injection study: Stoner *et al*., 1973. Groups of 20 A/He mice (male and female) received 8 or 12 injections of estradiol mustard at three dose levels (total doses: 0.48, 1.2, 1.6 g/kg bw). The experiment was terminated 24 weeks after the first injection. Lung tumor rates were 11/15, 19/19, and 19/19, with 2.8, 3.6, and 5 lung tumors per mouse for the low-, mid-, and high-dose groups, respectively. Of the 77 males and 77 females injected with vehicle alone surviving 24 weeks, 28% males and 20% females developed lung tumors, with 0.24 and 0.2 lung tumors per mouse (IARC, 1975).

Other relevant data

No genotoxicity data were located for estradiol mustard. However, the compound is expected to be an alkylating agent as it contains a nitrogen mustard functional group. Nitrogen mustards are known for their ability to alkylate DNA and proteins.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** as estradiol mustard has been shown to induce lymphoma, lung tumors, and sarcoma of the myocardium in both male and female mice. Sarcoma of the myocardium is a rare tumor in this strain of mice; it has not occurred in any of the more than 500 male and 500 female historical control mice. It is noteworthy that these tumors were observed in spite of the less-than-lifetime exposure (one year). Estradiol mustard also induced fibroadenoma of the mammary gland in male rats in a dose-related trend; however, the difference in the incidence rates was not statistically significant. Mammary gland tumors in male rats are considered rare. The concern is reinforced by the observations that estradiol mustard induced lung tumors in the strain A mouse, and the chemical is structurally related to nitrogen mustard, a known alkylating agent of DNA.

There is **LOW** level of **concern over the extent of exposure** as the chemical is not listed in the Physicians' Desk Reference (1997) and is not believed to be currently used for cancer treatment.

References

International Agency for Research on Cancer (IARC, 1975). *IARC monographs on the evaluation of the carcinogenic risk of chemicals to man: Some aziridines, N-, S- & O-mustards and selenium.* Vol. 9. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of the carcinogenic risks to humans. Overall evaluations of carcinogenicity: An updating of IARC Monographs Volumes 1 to 42.* Supplement 7: p. 68. IARC, Lyon, France.

National Cancer Institute (NCI, 1978). Bioassay of estradiol mustard for possible carcinogenicity. National Institutes of Health, US Department of Health, Education and Welfare. Carcinogenesis technical report series No. 59.

Physicians' Desk Reference (PDR, 1997). Physicians' Desk Reference. 51st Edition. Medical Economics Company, Inc. at Montvale, NJ.

Stoner GD, Shimkin MB, Kniazeff AJ, Weisburger JH, Weisburger EK, Gori GB (1973). Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in Strain A mice. *Cancer Res* **33**:3069-3085.

CARCINOGENICITY DATA SUMMARY: PIVALOLACTONE

Pivalolactone (3,3-dimethyl-2-propiolactone; pivalic acid lactone; CAS No. 1955-45-9) is primarily used as a chemical intermediate in the production of sulfur-containing pivalic acid derivatives (HSDB, 1995) and in the production of copolymers for sutures and prosthetic devices, and for the blocking and grafting of copolymers such as acrylics, isoprene, butadiene, and ethylenemethacrylic acid-vinyl acetate polymer (NCI, 1978).

Pivalolactone has not been produced or sold commercially in the US as of 1979 (HSDB, 1995; NCI, 1978). No data were available regarding US imports (HSDB, 1995). Individuals at risk for exposure may include workers involved in the copolymer production, although no numbers are available.

Carcinogenicity Data available:

Epidemiological studies

No data regarding the carcinogenicity of pivalolactone to humans were located in the literature.

Animal bioassays

1. Mouse long-term oral gavage studies: NCI, 1978. $B6C3F_1$ mice (50/sex/dose, but only 20 control animals) were administered pivalolactone by gavage at doses of 0, 75, or 150 mg/kg-day for 102 weeks. No statistically significant increase in tumor incidence was observed among the treated mice, nor were any rare or unusual tumors observed. No compound-related weight loss or clinical signs were observed in the mice, suggesting that a maximum tolerated dose (MTD) was not achieved. It was also noted that pivalolactone decomposes relatively rapidly in water. The test compound also contained boron trifluoride and tribenzylamine as stabilizers, but the NTP reported there were no indications in the literature of their carcinogenesis. The NCI reported that this study provided no evidence for the carcinogenicity of pivalolactone in $B6C3F_1$ mice.

1. Rat long-term oral gavage studies: NCI, 1978. Fischer 344 rats (50/sex/dose, but only 20 control animals) were administered pivalolactone by gavage at doses of 0, 150, or 300 mg/kg-day for 103 weeks. Both male and female rats showed evidence of a dose-related increase of hyperplasia, squamous-cell papillomas, and squamous-cell carcinomas of the forestomach. The incidences of squamous-cell carcinomas were 0/19, 1/49, and 7/48 in males and 0/20, 0/50, and 2/50 in females for the control, low-, and high-dose groups, respectively. The combined incidence of papillomas and carcinomas was statistically increased in high-dose male and female rats (males: 0/19, 6/49, 21/48 ($p<0.001$); females: 0/20, 2/50, 11/50 ($p=0.017$), for control, low-, and high-dose groups, respectively). The test compound also contained boron trifluoride and tribenzylamine as stabilizers, but the NTP reported there were no indications in the literature of their carcinogenesis. The NCI reported that under the conditions of this bioassay, pivalolactone was carcinogenic to both male and female Fischer 344 rats.

1. Mouse short-term intraperitoneal studies: Maronpot et al., 1986. A/St mice (10/sex/dose) were administered pivalolactone in tricaprylin intraperitoneally 3 times/week for 6-8 weeks at doses of 25, 50, or 100 mg/kg, then sacrificed at 24 weeks. Twenty percent of females in the high-dose group, 10% of males in the mid-dose group, and 11 and 22% of males and females, respectively, in the low-dose group developed pulmonary alveolar/bronchiolar adenomas. The incidences among 120 male and 80 female untreated controls were 2 and 8%, respectively, while the incidences among 60 male and female vehicle controls were 13 and 11%, respectively. In another experiment, male A/J mice (30/group) were similarly administered 16, 40, or 80 mg/kg pivalolactone intraperitoneally. The lung tumor incidences in increasing dose groups were 13, 16, and 23%. These values were below a 33% incidence observed in untreated control mice and 27% incidence observed in vehicle control mice.

Other relevant data

Pivalolactone was reported to give a positive result in a reverse mutation test in *Serratia marcescens*, but not *Escherichia coli* (Dean, 1972). Pivalolactone was found to be mutagenic to *Salmonella typhimurium* strain TA100 (in 3 of 3 laboratories) and TA1535 (in 1 of 3 laboratories) (Dunkel et al., 1985). Mutagenicity was considered

'clear' or 'questionable' in *Escherichia coli* in 3 of 3 laboratories. Pivalolactone bears a structural resemblance to beta-propiolactone, a direct-acting carcinogen which has been listed under Proposition 65 (IARC, 1974).

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH** level of **carcinogenicity concern** regarding pivalolactone because of evidence for induction of forestomach tumors in both sexes of rats. There is also some evidence of increased lung tumors in Strain A mice administered pivalolactone intraperitoneally. This level of concern is reinforced by findings of genotoxic effects in bacterial mutagenesis assays and structural similarity to a known Proposition 65 carcinogen, beta-propiolactone.

There is a **LOW level of exposure concern** regarding pivalolactone since it appears not to be produced or sold in the US. Some laboratory or experimental use of the chemical may occur and result in occupational exposure.

References

Dean BJ (1972). The mutagenic effects of organophosphorus pesticides on micro-organisms. *Arch Toxicol* **30**(1):67-74.

Dunkel VC, Zeiger E, Brusnick D, McCoy E, McGregor D, Mortelmans K, Rosenkranz HS, Simmon VF (1985). Reproducibility of microbial mutagenicity assays. II. Testing of carcinogens and noncarcinogens in *Salmonella* and *Escherichia coli*. *Environ Mutagen* **7**(Suppl 5):1-248

Hazardous Substances Data Bank (HSDB, 1995). National Library of Medicine. Bethesda, MD.

International Agency for Research on Cancer (IARC, 1974). *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man: Some Aromatic Amines, Hydrazine, and Related Substances, N-Nitroso Compounds, and Miscellaneous Alkylating Agents*. Vol. 4. IARC, Lyon, France.

Maronpot RR, Shimkin MB, Witschi HP, Smith LH, Cline JM (1986). Strain A mouse pulmonary tumor test results for chemicals previously tested in the National Cancer Institute carcinogenicity tests. *J Nat Cancer Inst* **76**(6): 1101-1112.

National Cancer Institute (NCI, 1978). Bioassay of pivalolactone for possible carcinogenicity. CAS No. 1955-45-9. Technical Report Series No. 140. US Department of Health, Education, and Welfare. Public Health Service. National Institutes of Health.

CARCINOGENICITY DATA SUMMARY: 2,4,6-TRIMETHYLANILINE AND ITS HYDROCHLORIDE

2,4,6-Trimethylaniline (mesidine; CAS No. 88-05-1) is produced commercially by the reduction of nitromesitylene, and is believed to be produced only in Japan, Switzerland, and France (IARC, 1982). There is no evidence that this chemical has ever been produced in commercial quantities in the U.S. Although three dyes can be prepared from 2,4,6-trimethylaniline (IARC, 1982), only one, Acid Blue 129, has been produced in commercial quantities in the U.S., and its production was last reported in 1971 (IARC, 1982). In addition, 2,4,6-trimethylaniline is believed to be used as an intermediate in the manufacture of Trimecaine, a local anesthetic produced and marketed for use in the former Soviet Union and Eastern Europe. It is not utilized in the U.S. or western Europe (IARC, 1982).

IARC (1982) reviewed 2,4,6-trimethylaniline and concluded that the evidence of carcinogenicity in experimental animals was inadequate. The IARC review did not include the genotoxicity studies reported by Kugler-Steigmeier *et al.* (1989), or Yoshimi *et al.* (1988).

Carcinogenicity Data available:

Epidemiological studies

No studies on the long-term effects of human exposure to 2,4,6-trimethylaniline or its hydrochloride have been reported.

Animal bioassays

1. Mouse feeding studies (18 months): Weisburger *et al.*, 1978. Experimental groups consisted of 25 male and 25 female Albino CD-1 mice. Low-dose and high-dose mice were fed 500 and 1,000 mg of 2,4,6-trimethylaniline hydrochloride/kg of diet, respectively, for three months. Low-dose and high-dose mice were then fed adjusted doses of 300 or 600 mg of 2,4,6-trimethylaniline hydrochloride/kg of diet, respectively, for another 15 months. Incidences of hepatomas in male mice were 0/14 in simultaneous controls, 7/99 in pooled controls, 5/15 in low-dose mice ($p<0.05$; p values by Fisher's exact test relative to matched controls except where noted), and 9/13 in high-dose mice ($p<0.025$). Among female mice the incidences of hepatomas in these same groups were 1/15, 1/102, 1/12, and 12/16 ($p<0.025$), respectively. Vascular tumors were also increased in high-dose males. The respective vascular tumor incidences were 2/14, 5/99, 1/15, and 4/13 ($p<0.025$). The authors concluded that 2,4,6-trimethylaniline was an effective carcinogen in male and female mice. IARC (1982) noted that the poor survival and the scant detail in the reporting make evaluation of the study difficult.

2. Male rat feeding study (18 months): Weisburger *et al.*, 1978. Two groups of 25 low-dose and high-dose male CD rats were fed 250 and 500 mg of 2,4,6-trimethylaniline hydrochloride/kg of diet, respectively, for three months. Low-dose and high-dose rats were then fed adjusted doses of 125 or 250 mg of 2,4,6-thrimethylaniline hydrochloride/kg of diet, respectively, for another 15 months. Significantly increased incidences of liver neoplasms (both hepatocellular carcinomas and cholangiocarcinomas), adenomas and adenocarcinomas of the lung, and stomach tumors were observed. The corresponding incidences in matched controls, pooled controls, low-dose rats, and high-dose rats were 0/16, 2/111, 4/20 ($p<0.025$ when compared with pooled controls only), and 8/21 ($p<0.025$) for liver tumors; 0/16, 2/111, 5/20 ($p<0.05$), and 8/21 ($p<0.025$) for lung tumors, and 0/16, 2/111, 0/20, and 3/21 ($p<0.05$ when compared with pooled controls only) for stomach tumors. The authors concluded that 2,4,6-trimethylaniline was an effective carcinogen in male rats. IARC (1982) noted the poor survival of the control group and the inadequate reporting of the data.

3. Male rat feeding study (2 years): Russfield *et al.*, 1973. Fifty male Sprague-Dawley rats were fed an unspecified amount of 2,4,6-trimethylaniline for two years. Seven experimental animals developed cholangiocarcinomas, while 2/111 control animals developed cholangiocarcinomas.

Several older studies have reported the occurrence of various tumors, including a hepatoma and pituitary tumors in rats fed 4000 mg of 2,4,6-trimethylaniline/kg (Kovi and Morris, 1976; 1977), pulmonary adenomas in mice (Gargus *et al.*, 1968) and a liver tumor in a single rat fed 844 mg (= 4200 mg/kg assuming lifetime average body weight of

200g) of 2,4,6-trimethylaniline (Morris and Wagner, 1964). The latter tumor was categorized histologically as a slow-growing, but metastatic, "minimal deviation hepatoma".

Other relevant data

Yoshimi *et al.* (1988) examined the genotoxicity of 2,4,6-trimethylaniline via a DNA repair test with rat hepatocytes and found that 2,4,6-trimethylaniline did induce DNA repair. Zimmer *et al.* (1980) found that 2,4,6-trimethylaniline and several other substituted anilines damaged the DNA of Chinese hamster lung fibroblast V79 cells. 2,4,6-Trimethylaniline was not mutagenic in *Salmonella typhimurium* (Zimmer *et al.*, 1980). Kugler-Steigmeier *et al.* (1989) performed a number of short-term genotoxicity tests and found that 2,4,6-trimethylaniline was weakly mutagenic in *Salmonella typhimurium*, mutagenic in the wing spot test in *Drosophila melanogaster*, and mutagenic in the 6-thioguanine resistance test in cultured fibroblasts. The authors concluded that genotoxicity seems to be a general property of aniline derivatives. A close structural analogue, 2,4,5-trimethylaniline, has also been shown to induce liver tumors in mice and rats, and is listed as "causing cancer" under Proposition 65. Other substituted anilines have also been shown to induce tumors in animals.

Webb *et al.* (1967) reported an increase in tissue DNA content and ^{14}C-thymidine incorporation (considered markers for cell proliferation) in rats fed 100 - 1000 ppm 2,4,6-trimethylaniline. These authors also noted nodular distortion of normal liver architecture, bile duct proliferation and fibrosis in treated animals, as did Lindstrom *et al.* (1969). Lindstrom *et al.* (1969) also demonstrated extensive (but only partly quantified) metabolism of 2,4,6-trimethylaniline to 3,5-dimethyl 2- and 4-amino benzoic acids, 2,6-dimethyl quinone and -hydroquinone, and conjugates. They found that 2,4,6-trimethylaniline induced methemoglobinemia, an indicator of the formation of an N-hydroxylated metabolite. Formation of N-hydroxylated metabolites and conjugates thereof is considered to be an important step in the mechanism of activation for various carcinogenic aromatic amines.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over 2,4,6-trimethylaniline and its hydrochloride. Tumors were observed at multiple sites in multiple studies with rats and mice. Although these studies suffer from significant design or reporting deficiencies, it appears likely that the effects described are real. The level of concern is strengthened by the observation of genotoxicity in multiple short-term tests, toxicity and metabolism data similar to those observed for aromatic amines known to be carcinogenic, and by structural analogies with these animal and human carcinogens.

There a **LOW** level of **concern over the extent of exposure** to 2,4,6-trimethylaniline. It is not produced in commercial quantities in the U.S., nor are products available in the U.S. that are based on this chemical. It was used commercially in the U.S. prior to 1971 in the manufacture of at least one dye product; no information on the potential for exposure through environmental routes as a result of such commercial activity has been identified. A search of the scientific literature between 1982 and 1997 did not identify any other information regarding the production or use patterns of this chemical. It is possible that 2,4,6-trimethylaniline may be derived by metabolism from certain azo dyes, including Ponceau 3R (formerly registered as FD&C Red #1). However, the extent of exposure to such dyes, and to their metabolites, is probably now small due to their limited current use.

References

Gargus JL, Paynter OE, Reese WH (1968). Utilization of newborn mice in the bioassay of chemical carcinogens. *Toxicol Appl Pharmacol* **12**:310.

International Agency for Research on Cancer (IARC, 1982). *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans; Some aromatic amines, antraquinones and nitroso compounds, and inorganic fluorides used in drinking water and dental preparations*. Volume 27, pp.177-188. IARC, Lyon, France.

Kovi J, Morris HP (1976). Ultrastructure of a mammosomatotrophic and a nonfunctional transplantable pituitary tumor induced in rats by 2,4,6-trimethylaniline. *J Nat Cancer Inst* **57**:197-205.

Kovi J, Morris HP (1977). Chromosome banding patterns of two transplantable pituitary tumors induced in rats by 2,4,6-trimethylaniline. *J Nat Cancer Inst* **58**:377-381.

Kugler-Steigmeier ME, Friederich U, Graf U, Lutz WK, Maier P, Schlatter C (1989). Genotoxicity of aniline derivatives in various short-term tests. *Mutat Res* **211**:279-289.

Lindstrom HV, Bowie WC, Wallace WC, Nelson AA, Fitzhugh OG (1969). The toxicity and metabolism of mesidine and pseudocumidine in rats. *J Pharm Exp Ther* **167**(2):223-234.

Morris HP, Wagner BP (1964). The development of "minimal deviation" hepatomas. *Acta Unio Int Contra Cancrum* **20**:1364-1366.

Russfield AB, Boger E, Homburger F, Weisburger EK, Weisburger JH (1973). Effect of structure on 7 methyl anilines on toxicity and on incidence of subcutaneous and liver tumors in Charles River rats. *Fed Proc* **32**:833.

Webb JM, Brouwer JB, Brouwer EA (1967). Cell proliferation in the rat liver: nucleic acid ratios and thymidine uptake as related to dose of a toxicant, mesidine. *Toxicol Appl Pharmacol* **10**:300-312.

Weisburger EK, Russfield AS, Homburger F, Weisburger JH, Boger E, Van Dongen CG, Chu KC (1978). Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J Environ Pathol Toxicol* **2**:325-356.

Yoshimi N, Sugie S, Iwata H, Niwa K, Mori H, Hashida C, Shimizu H (1988). The genotoxicity of a variety of aniline derivatives in a DNA repair test with primary cultured rat hepatocytes. *Mutat Res* **206**:183-191.

Zimmer D, Mazurek J, Petzold G, Bhuyan BK (1980). Bacterial mutagenicity and mammalian cell DNA damage by several substituted anilines. *Mutat Res* **77**:317-326.

CARCINOGENICITY DATA SUMMARY: 4-*bis*(2-HYDROXYETHYL)AMINO-2-(5-NITRO-2-THIENYL)-QUINAZOLINE

4-*Bis*(2-hydroxyethyl)amino-2-(5-nitro-2-thienyl)-quinazoline (BANTQ; CAS No. 33372-39-3) has been used as a research chemical in studies of carcinogenesis in laboratory animals and mutagenesis in bacteria. No other use has been identified.

Carcinogenicity Data available:

Epidemiological studies

No epidemiological studies of cancer rates in humans exposed to BANTQ were found in the literature.

Animal bioassays

1. Rat feeding studies: Cohen *et al*., 1976. BANTQ was administered in feed to 20 male and 28 female rats at a concentration of 0.05%. Male rats were treated for 50 weeks and then observed for another 2 weeks without further exposure before they were killed and examined for tumors. Females were treated for 15 weeks and then observed for another 9 weeks without further exposure before they were killed. Control groups of 20 males and 84 females were given the standard laboratory diet. Control males and control females were killed after 58 and 66 weeks, respectively. In males the incidence of adenocarcinomas of the mammary gland was 5/19. This is significantly increased above the incidence found in the controls (0/20, $p=0.02$). The incidence of intestinal tumors (leiomyosarcomas) in males was significantly increased above the incidence found in controls (12/20 vs. 0/20; $p<0.001$). In females the incidence of adenocarcinomas of the mammary gland was 28/28. This is significantly increased above the incidence in the controls (2/84, $p<0.001$). In untreated male rats, mammary adenocarcinomas and intestinal leiomyocarcinomas are rare tumors. In treated female rats, the incidence of mammary adenocarcinomas is unusually high for an experiment of such short duration.

Other relevant data

BANTQ was mutagenic in the *Salmonella typhimurium* mutagenesis test in the absence of metabolic activation (Wang *et al*., 1975).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over BANTQ because it produced an unusually high incidence of mammary gland adenocarcinomas in an unusually short time in female rats, and it produced high incidences of uncommon tumors (mammary adenocarcinomas and intestinal leiomyosarcomas) in male rats. The level of concern is supported by data demonstrating that BANTQ is mutagenic in bacteria.

There is **NO IDENTIFIED CONCERN over the extent of exposure** to BANTQ because it has no identified use other than limited use as a research chemical.

References

Cohen SM, Erturk F, Bryan GT (1976). Comparative carcinogenicity of 5-nitrothiophenes and 5-nitrofurans in rats. *J Nat Cancer Inst* **57**:277-282.

Wang CY, Muraoka K, Bryan GT (1975). Mutagenicity of nitrofurans, nitrothiophenes, nitropyroles, nitroimidazole, aminothiophenes, and aminothiazoles in *Salmonella typhimurium*. *Cancer Res* **35**:3611-3517.

CARCINOGENICITY DATA SUMMARY: 3-(CHLOROMETHYL)PYRIDINE HYDROCHLORIDE

3-(Chloromethyl)pyridine hydrochloride (CAS No. 6959-48-4) is a research chemical which has been proposed for use as a chemical intermediate in the synthesis of agricultural, pharmaceutical, and veterinary chemicals (NCI, 1978). 3-(Chloromethyl)pyridine hydrochloride was selected by NCI (1978) for carcinogenicity evaluation because, although this chemical was neither manufactured in nor imported into the U.S., it was felt that this agent could become a widely used industrial intermediate.

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to 3-(chloromethyl)pyridine hydrochloride were found.

Animal bioassays

1. Rat long-term gavage studies: NCI, 1978. Groups of 50 male and 50 female F344 rats were administered 75 or 150 mg/kg 3-(chloromethyl)pyridine hydrochloride by gavage three times per week for 103 weeks. Because of early mortality, the high-dose rats were dosed for only 83 weeks. Vehicle controls consisted of 20 male and 20 female rats. All surviving rats were killed at 104 weeks. Squamous cell lesions (including carcinomas, papillomas, and hyperplasia) of the stomach were observed in both sexes. The combined incidence of squamous-cell papillomas and carcinomas of the stomach in male rats was 0/19 in the controls, 1/47 in the low-dose group, and 3/50 in the high-dose group. The Fisher exact test results were not significant when compared with concurrent controls. However, comparison of the incidence of these tumors in the high-dose males with that in 99 historical gavage vehicle-controls showed that the probability that 3 such tumors occurred by chance, given that none were observed in the historical controls, is very small ($p=0.014$). There was one squamous-cell carcinoma in the high-dose females (1/48) compared with none in the 100 historical gavage vehicle-control female rats. NCI concluded that under the conditions of the bioassay, 3-(chloromethyl)pyridine hydrochloride was carcinogenic in male F344 rats.

2. Mouse long-term gavage studies: NCI, 1978. Groups of 50 male and 50 female $B6C3F_1$ mice were administered 100 or 200 mg/kg 3-(chloromethyl)pyridine hydrochloride by gavage three times per week for 102 weeks. Because of early mortality, the high-dose mice were dosed for only 81 weeks. Vehicle controls consisted of 20 male and 20 female mice. All surviving mice were killed at 104 weeks. There were dose-related increases in squamous-cell papillomas and carcinomas of the stomach in the low- and high-dose male (2/43, 10/47) and female (1/45, 5/48) mice. No such tumors were found in the vehicle controls of either sex. The incidence of stomach tumors in the high-dose males was significantly higher than in the control males. Also, a life-table analysis of the incidence of stomach tumors in males indicated a significant ($p=0.003$) dose-response relationship. Comparison of the incidence of these tumors in the high-dose males and females with those observed in 100 historical controls of each sex showed that the probability that their occurrence was due to chance is very small ($p<0.001$). NCI concluded that under the conditions of the bioassay, 3-(chloromethyl)pyridine hydrochloride was carcinogenic in male and female $B6C3F_1$ mice, producing papillomas and carcinomas at the site of application, the stomach.

Other relevant data

3-(chloromethyl)pyridine hydrochloride was reported to be positive in cell transformation in the RLV F344 rat embryo assay and prophage induction assay in *Escherichia coli* (DeMarini *et al*., 1992). The chemical was also mutagenic in the L5178 TK+/TK- mouse lymphoma forward mutation assay (McGregor *et al*., 1987; Mitchell *et al*., 1988) and the Ames-*Salmonella* assay (Ashby *et al*., 1988; Claxton *et al*., 1987; Dunkel *et al*., 1985). Sex-linked recessive lethal mutations were reported in one study of *Drosophila melanogaster* (Foureman *et al*., 1994).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over 3-(chloromethyl)pyridine hydrochloride because it induced a dose-related increase of malignant and benign stomach tumors in male mice. Although the increases in stomach tumors in male rats and female mice are not statistically significant compared with the concurrent controls, they are statistically significant when compared with historical controls. Because stomach tumors are rare in the strain of animals tested and were found in both sexes of dosed mice and rats, they are considered to be related to the administration of 3-(chloromethyl)pyridine hydrochloride. The level of concern is also supported by the positive mutagenicity data.

There is **NO IDENTIFIED concern over the extent of exposure** to 3-(chloromethyl)pyridine hydrochloride. As of 1978, this chemical was neither manufactured in nor imported into the U.S. NCI (1978) selected 3-(chloromethyl)pyridine hydrochloride for carcinogenicity evaluation because it was felt that this chemical could become a widely used industrial intermediate.

References

Ashby J. Tennant RW (1988). Chemical structure, *Salmonella* mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the US NCI/NTP. *Mutat Res* 204:17-115.

Claxton LD, Dearfield KL, Spanggord RJ, Riccio ES, Mortelmans K (1987). Comparative mutagenicity of halogenated pyridines in the *Salmonella typhimurium*/mammalian microsome test. *Mutat Res* 176:185-198.

DeMarini DM, Brooks HG (1992). Induction of prophage lambda by chlorinated organics: detection of some single-species/single-site carcinogens. *Environ Mol Mutagen* 19:98-111.

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, Resenkranz HS, Simmon VF (1985). Reproducibility of microbial mutagenicity assay: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ Mutagen* 7(5)1-19.

Foureman P, Mason JM, Valencia R, Zimmering S (1994). Chemical mutagenesis testing in Drosophila. X. Results of 70 coded chemicals tested for the National Toxicology Program. *Environ Mol Mutagen* 23:208-227.

McGregor DB, Martin R, Cattanach P, Edwards I, McBride D, Caspary WJ (1987). Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay to coded chemicals. I: Results for nine compounds. *Enivron Mutagen* 9:143-160.

Mitchell AD, Rudd CJ, Caspary WJ (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: intralaboratory results for sixty-three coded chemicals tested at SRI International. *Environ Mol Mutagen* 12(13):37-101.

National Cancer Institute (NCI, 1978). *Bioassay of 3-(chloromethyl)pyridine hydrochloride for possible carcinogenicity*. Carcinogenesis Technical Report Series NCI-TR-95, Department of Health Education, and Welfare, National Institutes of Health. DHEW Publication. No. (NIH) 78-1345.

CARCINOGENICITY DATA SUMMARY: DIMETHYLDIAZENE-1-OXIDE

Dimethyldiazene-1-oxide (MAM; methylazoxymethane; azoxymethane; z-methyl-O,N,N-azoxymethane; CAS No. 25843-45-2) is used as a model carcinogen in research laboratories. It acts as a methylating agent after metabolic activation.

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to MAM were identified.

Animal bioassays

A large number of carcinogenicity studies have been reported in which animals were treated with MAM, often as part of mechanistic studies. Review of CCRIS (1994), PHS 149 (1972-1992) and RTECS (1997) identified 26 positive bioassay results, and 9 non-positive results. Species tested were mice, rats, guinea pigs and Syrian golden hamsters; elevated tumor incidences at various sites have been noted in some studies in each of these species. The size, design and reporting of these studies is variable, and in several cases reflects an intent to examine questions of mechanism and structure/reactivity comparisons rather than following a standard bioassay protocol. Accounts of some of the more recent studies are given below.

1. Rat oral and injection studies (33 weeks): Druckrey, 1973 (reviewing several earlier reports by Druckrey *et al.*). BD rats subcutaneously injected with 6 mg/kg MAM five times a week for 33 weeks suffered a 95% incidence of adenocarcinomas of the colon and rectum. Some liver tumors were also observed in another experiment in which oral administration was used. Experiments to demonstrate transplacental carcinogenesis were also described in this review. Pregnant rats received single intravenous injections of 20 mg/kg MAM on the 15^{th} or 22^{nd} day of gestation. In the group exposed on the 22^{nd} day, 5 kidney tumors and 2 nervous-system derived tumors were observed among 34 surviving fetuses; however, no tumors were observed among 175 survivors when treatment was given on the 15^{th} day.

2. Rat long-term drinking water study (90 weeks): Lijinsky *et al.*, 1985. Groups of 20 male F344 rats were given MAM (and, in parallel groups in the same study, related compounds with one or two ethyl groups in place of methyl) in their drinking water at concentrations of 10 and 40 mg/L, for total doses of 30 and 120 mg. Control groups received plain drinking water. Treatment continued for 30 weeks. MAM was carcinogenic at both doses (16/20 and 17/20 animals, respectively, developed neoplasms of the liver, with hepatocellular carcinomas and neoplastic nodules being the most prevalent). Liver hemangiosarcomas and tumors in the kidney and colon were also observed in treated animals but not in controls.

3. Rat gavage study (70 weeks): Lijinsky *et al.*, 1987. Groups of 16 male F344 rats were given 1 mg MAM by gavage (in 0.1 ml corn oil), twice a week for 20 weeks. Observations continued until all treated animals died: no treated animals survived to the 70^{th} week of the study. Most animals developed colon tumors and several had mesenchymal tumors of the kidney and tumors of the Zymbal gland. No corn-oil treated control rats had died by the time the last MAM-treated rat had died of tumors.

4. Hamster gavage study (60 weeks): Lijinsky *et al.*, 1987. Male Syrian golden hamsters were given MAM in deionized water (concentration = 7.5 mg/ml), by gavage with 0.2 ml once a week for 4 weeks, 0.1 ml twice a week for 6.5 weeks or 0.1 ml once a week for 20 weeks. Observations continued until all treated animals died: no treated animals survived to the 60^{th} week of the study. Mortality was severe in the two higher dose groups. A near 100% incidence of liver tumors occurred in all treated groups (6/6, 13/14, 10/10).

5. Rat injection study (30 weeks): Tatsuta *et al.*, 1992. Groups of 25 male Wistar rats were given MAM by subcutaneous injection once a week for 10 weeks, at doses of 7.4 mg/kg body weight, in combination with normal or low-protein diets. A control group was not included (but background incidence of colon tumors is low in this strain of rat). Treated rats fed a normal protein diet had a 44% (8/18) incidence of colon tumors.

The incidence of colon tumors was much higher in MAM treated rats fed low and very low protein diets: 88% (15/17) and 100% (17/17), respectively.

Other relevant data

MAM is an alkyl azoxyalkane (which is isomeric with the corresponding dialkyl nitrosamine). It is an indirect acting methylating agent. Positive genotoxicity data have been reported in various tests, including the *Salmonella* reverse mutation test *in vitro*, a host-mediated assay, and sex-linked lethal mutations in *Drosophila melanogaster*. It was negative for cell transformation in BALB/C-3T3 cells [studies cited in RTECS (1997) and NIOSHTIC (1996)]. Studies *in vivo* indicate that MAM induces G-A transitions in codon 12 of the K-ras gene, as detected in mouse colon tumors following MAM administration.

A proposed mechanism for metabolic activation involves oxidation, possibly by mixed function oxidases, with the eventual formation of a methyldiazonium ion (Lijinsky *et al.*, 1985). The observation by Druckrey (1973) that the fetus was only sensitive to MAM transplacental carcinogenesis late in gestation was interpreted as indicating that the compound itself was not the proximate carcinogen, but that metabolic activation was required. At the earlier dosing time in that experiment, the necessary enzyme activities had not appeared in the fetus. MAM was not teratogenic at any stage of pregnancy in rats, whereas a presumed metabolite, methylazoxymethanol, was both teratogenic and carcinogenic at all stages examined by Druckrey (1973). MAM has been studied as a probable intermediate in the carcinogenic action of 1,2-dimethylhydrazine, a carcinogen listed under Proposition 65. Azoxyalkanes are reported to be short-lived intermediates in carcinogenic action. Lijinsky *et al.* (1985, 1987) and Druckrey (1973) both report extensive investigations of large numbers of related hydrazines, dialkylnitrosamines and azoxyalkanes, many of which are related both by structural analogy and metabolic conversion: many of these compounds have also been demonstrated to have carcinogenic activity.

Druckrey (1973) stated that MAM-induced rat colon tumors display many features of human colorectal cancer (carcinoma), and considered that the subcutaneous injection protocol described above (which also produces a high incidence of tumors in mice) is the most reliable animal model for studies of colon cancer. Carcinogen-treated rats develop foci of aberrant crypts in the colon (ACFs) that may be preneoplastic lesions, although one study (Cadneri *et al.* 1985) in which 1,2-dimethylhydrazine was the carcinogen found no association between the number of ACFs, ACF multiplicity, and the presence of tumors. MAM-induced intestinal tumorigenesis has been used as a model to study the chemoprotective activity of other compounds as well as the effect of fats, protein, and other dietary aspects on colon cancer.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over MAM since tumors were observed in multiple studies by various routes in four species. The concern is reinforced by positive genotoxicity studies, structural analogies with other known carcinogens, and by the demonstration of metabolic processes leading to reactive, genotoxic and carcinogenic metabolites.

There is **NO IDENTIFIED CONCERN over the extent of exposure** to MAM. This chemical is used only as a model carcinogen in research laboratories, resulting in very limited human exposure.

References

Cadneri G, Giannini A, Lancioni L, Luceri C, Biggeri A, Dolara P (1985). Characterization of aberrant crypt foci in carcinogen-treated rats: association with intestinal carcinogenesis. *Br J Cancer* **71**(4):763-769.

Chemical Carcinogenesis Research Information System (CCRIS, 1994). Record 28543-45-2.

Druckrey H (1973). Specific carcinogenic and teratogenic effects of 'indirect' alkylating methyl and ethyl compounds, and their dependency on stages of ontogenic developments. *Xenobiotica* **3**(5):271-303.

Lijinsky W, Saavedra JE, Reuber MD (1985). Organ-specific carcinogenesis in rats by methyl- and ethyl-azoxyalkanes. *Cancer Res* **45**:76-79.

Lijinsky W, Kovatch RM, Riggs CW (1987). Carcinogenesis by nitrosodialkylamines and azoxyalkanes given by gavage to rats and hamsters. *Cancer Res* **47**:3968-3972.

National Institute for Occupational Safety and Health (NIOSHTIC, 1996): NIOSHTIC Database, Issue 96-1 (February, 1996).

Public Health Service (PHS 149, 1972-1992). U.S. Department of Health, Education and Welfare / Department of Health and Human Services, National Cancer Institute, Bethesda, MD.

Registry of Toxic Effects of Chemical Substances (RTECS, 1997). Database produced by the U.S. National Institute for Occupational Safety and Health. Version date 1/97: RTECS number PA2975000.

Tatsuta M, Iishi H, Baba M, Taniguchi H (1992). Enhanced induction of colon carcinogenesis by MAM in Wistar rats fed a low-protein diet. *Int J Cancer* **50**:108-111.

CARCINOGENICITY DATA SUMMARY: N´-ETHYL-N-METHYL-N-NITROSOUREA

N´-Ethyl-N-methyl-N-nitrosourea (EMNU; 1-nitroso-1-methyl-3-ethylurea; CAS No. 72479-13-1) is an alkyl-nitrosourea and has been used by researchers as a model carcinogen.

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Rat 30-week drinking water studies: Lijinsky and Saavedra, 1989. Groups of 12 male and 12 female Fischer 344 rats were exposed to EMNU in drinking water, 5 days a week for 30 weeks. The total doses received by the high- and low-dose groups were 1.2 mmol/rat and 0.6 mmol/rat, respectively. Groups of 24 male and 24 female rats were used as controls. Dose-related increases of tumors of the nervous system were observed for both male (7/12 for low-dose and 10/12 for high-dose) and female rats (6/12 for low-dose and 11/12 for high-dose). No tumors of the nervous system were observed in male or female controls. In addition, lung tumors were observed among the exposed males (4/12 for low-dose and 1/12 for high-dose) and kidney tumors were observed among the exposed females (4/12 for low-dose and 1/12 for high-dose). No lung or kidney tumors were observed in the male and female controls.

2. Rat 30-week gavage studies: Lijinsky and Saavedra, 1989. Groups of 12 male and 12 female Fischer 344 rats were administered EMNU by gavage twice a week for 30 weeks. The total doses to two groups of male rats were 1.2 and 0.6 mmol, and the total doses to two groups of female rats were both 0.6 mmol. Significantly elevated incidences of tumor of the nervous system were observed in the exposed males (7/12 for low-dose and 9/12 for high-dose) and exposed females (3/12 and 8/12). No tumors of the nervous system were observed in the male (0/12) and female controls (0/12). Tumors of the thymus (9/12) and forestomach (2/12) were also observed in one of the two groups of exposed female rats. None of these tumors were observed in the female control rats (0/12).

Other Relevant Data:

Alkylnitrosoureas are powerful alkylating agents and have been shown to cause a broad spectrum of tumors in experimental animals (Lijinsky, 1992). EMNU was mutagenic in *S. typhimurium* strain TA1535, and was positive in the prophage induction assay (Lijinsky *et al.*, 1987).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** as EMNU has been shown to induce tumors of the nervous system and lung tumors in male rats and tumors of the nervous system, kidney tumors, and tumors of the thymus in female rats even though the dosing period was relatively short. The concern is supported by the positive mutagenicity data and the fact that many alkylnitrosoureas are carcinogenic to laboratory animals.

There is **NO IDENTIFIED CONCERN over the extent of exposure** of EMNU, as it is used solely as a model carcinogen for research purposes, and is not known to occur naturally.

References

Lijinsky W (1992). *Chemistry and biology of N-nitroso compounds*. Cambridge Monographs on Cancer Research. Cambridge University Press.

Lijinsky W, Saavedra JE (1989). Carcinogenesis in rats by nitrosodialkylureas containing methyl and ethyl groups given by gavage and in drinking water. *J Toxicol Environ Health* **28**:27-38.

Lijinsky W, Elespuru RK, Andrews AW (1987). Relative mutagenic and prophage-inducing effects of mono- and di-alkyl nitrosoureas. *Mutat Res* **178**:157-165.

CARCINOGENICITY DATA SUMMARY: N-ETHYL-N-NITROSOBUTYLAMINE

N-Ethyl-N-nitrosobutylamine (CAS No. 4549-44-4) is used as a chemical in laboratories for inducing esophageal cancer in test animals.

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Rat drinking water study: Mandard *et al*., 1984. Eighty male and female Wistar rats were exposed to N-ethyl-N-nitrosobutylamine via drinking water for the lifetime of the animals. During the first 4 weeks, the rats were exposed to 10 mg/kg of N-ethyl-N-nitrosobutylamine, the dosage was reduced to 5 mg/kg for the rest of the experiment. Forty male and female Wistar rats were used as controls. Among the animals exposed to N-ethyl-N-nitrosobutylamine, 29% developed carcinoma of the esophagus, 52% developed angiosarcoma of the liver, and 22% developed hepatocarcinoma. The first appearances of esophageal carcinoma and liver carcinomas were 13 and 14 weeks, respectively. None of these tumors were observed in the controls.

2. Rat oral study: Thomas *et al*., 1969. Thirty rats were exposed to N-ethyl-N-nitrosobutylamine via the oral route (5 mg/kg or 10 mg/kg), seven times a week. Total doses applied to the two groups of rats were 1.0 and 1.6 g/kg. All 30 exposed animals developed esophagus tumors; the mean latency periods for the high-dose and low-dose groups were 200 days and 240 days, respectively.

3. Rat drinking water study: Sons *et al*., 1985. Fifty male Wistar rats were exposed to N-ethyl-N-nitrosobutylamine via drinking water. Eight male rats were kept under the same experimental conditions, but without carcinogen exposure. All animals were killed after 93 days of exposure. A total of 95 esophageal tumors were identified in the exposed rats, and no such tumor was observed among the controls.

4. Rat rectal carcinogenicity study: Schmähl, 1970. N-ethyl-N-nitrosobutylamine was given to 40 male Sprague-Dawley rats by rectal application. A dose of 50 mg/kg/week was applied for 20 weeks. Beside toxic liver injuries, there were 8 malignant and 7 benign liver tumors. Nine rats showed papillomas and hyperkeratoses of the esophagus.

5. Rat intravenous injection study: Thomas *et al*., 1969. Fifteen rats were injected with N-ethyl-N-nitrosobutylamine at 25 mg/kg, once a week. The mean total dose was 1 g/kg. Esophagus tumors were observed in nine rats (60%) with a mean latency period of 363 days.

6. Mouse drinking water studies: Schmähl *et al*., 1963. N-Ethyl-N-nitrosobutylamine was given to 18 male and female DBA mice via drinking water. The daily dose to the exposed animals was 10 mg/kg and total mean doses were 2360±520 mg/kg. After 236 days, 14 out of 14 surviving mice developed squamous cell carcinoma of the esophagus. 20 mice were used as controls and no tumors were observed in this group at 260 days.

Other Relevant Data:

No genotoxicity and mutagenicity data of N-ethyl-N-nitrosobutylamine were identified. However, N-ethyl-N-nitrosobutylamine is a nitrosamine and is expected to alkylate macromolecules such as DNA and proteins. Many nitrosamines are potent mutagens and are carcinogenic to laboratory animals.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** as N-ethyl-N-nitrosobutylamine has been shown to increase the incidence of esophageal tumors and liver tumors in multiple studies. In mice, N-ethyl-N-nitrosobutylamine has been

shown to induce esophageal tumors in both males and females. The level of concern is supported by the structural-activity analysis of the compound.

There is **NO IDENTIFIED CONCERN over the extent of exposure** for N-ethyl-N-nitrosobutylamine. There is no known sources of exposure for this chemical except for laboratory use.

References

Mandard AM, Marnay J, Herlin P, Elie H, Tuyns AJ, Le Talaer JY (1984). Cancer de l'oesophage induit chez le rat Wistar par l'éthyl-N-butyl-nitrosamine. *Bull Cancer* (Paris) **71**:419-424.

Schmähl D (1970). Zur carcinogen wirkung von butyl-äthyl-nitrosamin bei rectaler applikation an ratten. *Zeitschrift Krebsforsch* **74**:110-111.

Schmähl D, Thomas C, Scheld G (1963). Carcinogen wirkung von äthyl-butyl-nitrosamin bei mäusen. *Naturwissenschaften* **50**(23):56.

Sons UH, Borchard F, Müller-jäh K, Sandmann H (1985). Accelerated tumor induction by distal esophageal constriction in the rat under the influence of N-ethyl-N-butyl-nitrosamine. *Cancer* **56**:2617-2621.

Thosmas VC, So BT (1969). Zur morphologie der durch N-nitroso-verbindungen erzeugten tumoren im oberen verdauungstrakt der ratte. *Arzneimittel-Forschung* **19**(7):1077-1081.

CARCINOGENICITY DATA SUMMARY: 4-ETHYLSULFONYLNAPHTHALENE-1-SULFONAMIDE

4-Ethylsulfonylnaphthalene-1-sulfonamide (ENS; ethyl-4-sulfonamidonaphthylsulfone; CAS No. 842-00-2) was originally considered as one of a class of potential pharmacological agents with anti-convulsant or diuretic properties (Santana, 1963). This was abandoned when evidence of bladder effects including hyperplasia and cancer were found in experimental animals. Current use appears to be limited to laboratory investigations.

Carcinogenicity Data available:

Epidemiological studies

No data regarding the carcinogenicity of ENS to humans have been located in the literature.

Animal bioassays

1. Mouse long-term feed studies: Bonser and Clayson, 1964. Sixty-five Ab x IF male and female mice (sex distribution not stated) were fed diet containing 0.01% ENS for up to 65 weeks or until moribund. Survival was 28/65 at 30 weeks and 14/65 at the end of the study. Among survivors to 30 weeks, 10 were found to have carcinomas of the bladder (9/16 females; 1/12 males). Although a control group was not included, the authors stated that "no spontaneous bladder tumor has ever been observed in the mouse colony at Leeds."

2. Mouse long-term feed studies: Clayson and Bonser, 1965. In two experiments, Ab x IF mice (original numbers not stated) were treated with 0.01% ENS in their diet for up to 69 weeks. Bladder carcinomas developed in 2/16 and 3/18 surviving male mice and 9/19 and 9/20 surviving female mice.

3. Mouse long-term feed studies: Clayson *et al.,* 1967. In an effort to understand the sex difference in susceptibility to the bladder carcinogenic effects of ENS, castrated male and sterilized female Ab x IF mice were treated with 0.01% ENS in their diet. No control group data were presented. Among mice surviving to 30 weeks, bladder carcinomas developed in 9/10 males and 4/6 female mice. These incidences were compared with those reported in Clayson and Bonser (1965). A second set of experiments were conducted using C57 x IF mice. Among mice surviving a diet of 0.01% ENS to 30 weeks (original numbers not reported), 2/13 males, 4/19 castrated males, 7/16 females, and 5/18 sterilized females developed bladder carcinomas. No other tumors were reported in mice surviving to 65 weeks.

4. Mouse long-term feed studies: Dzhioev *et al.,* 1969; some data also in Clayson and Wood, 1968. Groups of A x IF F1 mice of various sizes were treated for varying lengths of time with 0.01% ENS in the diet and examined for the development of bladder, liver, lung, and mammary gland tumors. No tumors were reported among groups of mice treated for 4 weeks (0.005% ENS; 9/sex) or 31 weeks (17 male, 25 female) with the ENS diet and sacrificed at the end of exposure. Among female mice surviving to 40-65 weeks on the ENS diet, 14/47 developed mammary tumors compared to 0/24 untreated mice surviving to 77 weeks. Six bladder carcinomas and one adenoma developed in 74 female mice surviving for 40 weeks on the ENS diet.

5. Mouse long-term feed study: Flaks *et al.,* 1973b. A group of 42 female A x IF mice was treated with ENS at 0.01% in the diet for up to 701 days along with 40 untreated mice. Bladder tumors developed in 9/42 treated mice relative to 0/40 untreated animals. Lung tumors developed in 7/42 ENS-treated mice (6 adenomas, 1 carcinoma) relative to 0/40 untreated animals ($p=0.007$). Mammary gland carcinomas developed in 18/42 ENS-treated mice relative to 13/40 untreated animals ($p=0.23$).

6. Mouse long-term feed study: Flaks and Clayson, 1975; Flaks *et al.,* 1973a, preliminary report. Female IF x C57 F1 mice (52/group) were fed diet containing 0, 0.01% ENS, 0.01% ENS plus ammonium chloride (1.0% in drinking water), or ammonium chloride alone for up to 648 days. Bladder tumors were observed in 19/50 mice treated with ENS alone, and in none of the mice in the other groups.

Other relevant data

ENS was tested for its ability to promote tumors of the urinary bladder in mice (Sen Gupta, 1962b). Female mice were first implanted in the bladder with paraffin pellets containing 2-amino-1-naphthol hydrochloride. Groups of mice were then maintained on normal diet (n=39) or diet containing 0.01% ENS (n=49). Among mice on the normal diet, 5 of 30 survivors to 40 weeks developed carcinomas of the urinary bladder, while 20 of 39 mice on the ENS diet developed bladder carcinomas.

This compound has been tested in a number of species for its ability to induce hyperplasia of the urinary tract epithelium (Sen Gupta, 1962a). Mice and rats (and possibly rabbits) were found to be susceptible to this effect, while guinea pigs and dogs appeared to be refractory, although the studies experimental design (small groups, relatively short durations) limit the conclusions which can be drawn.

Mechanistic studies using ENS have shown it to be an inhibitor of carbonic anhydrase activity in the kidney leading to the production of alkaluria, crystalluria and calculi in the urinary tract. The resulting hyperplasia is thought to have a role in the etiology of the bladder tumors. Supporting evidence includes the reversibility of the hyperplastic lesions following short-term exposure and the ability of ammonium chloride administered in the drinking water to prevent the formation of calculi and ultimately tumors (Flaks *et al.*, 1973a; Flaks and Clayson, 1975).

No short term tests of the genotoxicity of ENS in bacteria were identified.

Preliminary evaluation of carcinogenicity and exposure data

There is a **HIGH** level of **carcinogenicity concern** regarding 4-ethylsulfonylnaphthalene-1-sulfonamide because of evidence for the development of bladder tumors in both sexes of two strains of mice. There is also some evidence for the development of mammary and lung tumors in female mice.

There is **NO IDENTIFIED CONCERN regarding exposure** to 4-ethylsulfonylnaphthalene-1-sulfonamide because of its extremely limited use as a laboratory chemical.

References

Bonser GM, Clayson DB (1964). A sulphonamide derivative which induces urinary tract epithelial hyperplasia and carcinomas of the bladder epithelium in the mouse. *Br J Urol* **36**(1): 26-34.

Clayson DB, Bonser GM (1965). The induction of tumours of the mouse bladder epithelium by 4-ethylsulphonylnaphthalene-1-sulphonamide. *Br J Cancer* **19**: 311-316.

Clayson DB, Wood M (1968). Carcinogenicity tests in mice. 4-Ethylsulphonylnaphthalene-1-sulphonamide. British Empire Cancer Campaign for Research. 46th Annual Report, pp. 271-2.

Clayson DB, Pringle JAS, Bonser GM (1967). 4-Ethylsulphonylnaphthalene-1-sulphonamide: A new chemical for the study of bladder cancer in the mouse. *Biochem Pharmacol* **16**: 619-626.

Dzhioev FK, Wood M, Cowen DM, Campobasso O, Clayson DB (1969). Further investigations on the proliferative response of mouse bladder epithelium to 4-ethylsulphonylnaphthalene-1-sulphonamide. *Br J Cancer* **23**(4):772-780.

Flaks A, Hamilton JM, Clayson DB (1973). Effect of ammonium chloride on incidence of bladder tumors induced by 4-ethylsulfonylnaphthalene-1-sulphonamide. *J Nat Cancer Inst* **51**: 2007-8.

Flaks A, Hamilton HM, Clayson DB, Burch PRJ (1973a). The combined effect of radiation and chemical carcinogens in female A × IF mice. *Br J Cancer* **28**(3): 227-331.

Flaks A, Clayson DB (1975b). The influence of ammonium chloride on the induction of bladder tumours by 4-

ethylsulphonylnaphthalene-1-sulphonamide. *Br J Cancer* **31**: 585-7.

Santana S (1963). The use of rat bladder pouches to elucidate the mode of action of a chemical which induces hyperplasia in the bladder epithelium. *Br J Cancer* **7**(4): 715-718.

Sen Gupta KP (1962a). Hyperplasia of urinary tract epithelium induced by continuous administration of sulphonamide derivatives. *Br J Cancer* **16**:110-118.

Sen Gupta KP (1962b). Tumour-promoting action of 4-ethylsulphonylnaphthalene-1-sulphonamide. *Nature* **194**(4834):1185-6.

CARCINOGENICITY DATA SUMMARY: ICRF-159

ICRF-159 (1,2-bis(3,5-dioxopiperazine-1-yl)propane; CAS No. 21416-87-5), is an antineoplastic agent that has been used as an experimental drug to treat acute leukemias and tumors of the lung, breast, and colon. The agent has also been used for the treatment of severe psoriasis. ICRF-159 has no identified use other than as an experimental drug and has not been approved for use as a prescription drug in the U.S.

Carcinogenicity Data available:

Epidemiological studies

Two cases of cutaneous squamous-cell carcinomas developed among 70 patients treated with ICRF-159 for psoriasis (Horton *et al*., 1983). Both cases of cancer were detected approximately 3 years after ICRF-159 therapy began and occurred at a relatively young age on areas of skin not exposed to sunlight. Both patients had previous treatment with oral steroids, PUVA (psoralen plus long wavelength ultraviolet light), methotrexate, and topical therapy with tar, dithranol and steroids. However, the authors concluded that, with the exception of UV light, none of these previous treatments was known to cause cancer, and they stated that the duration of UV treatment was less than two months for each patient.

In a prospective trial study, 3 cases of acute myeloid leukemia (AML) were found in a five-year follow-up among 139 patients with colorectal cancer treated with adjuvant ICRF-159 (Gilbert *et al*., 1986). AML was not detected in any of the 133 patients in the control group.

There are several case reports of acute non-lymphocytic leukemia following ICRF-159 therapy. Two cases of acute myeloid leukemia were reported among patients receiving ICRF-159 for treatment of psoriasis (Horton *et al*., 1984). Joshi *et al*., (1981) reported that two cases of acute myelomonocytic leukemia (AMML) developed in patients after receiving ICRF-159 as the sole agent for treatment of cancer (1 carcinoma of the colon and 1 carcinoma of the pancreas). AMML developed in these rodents within 23 and 49 months of starting treatment. The authors comment that AMML has been reported following chemotherapy with many cytotoxic drugs and that use of ICRF-159 should be reconsidered as treatment for non-malignant conditions such as psoriasis. Bhavnani and Wolstenholme (1987) reported that a case of acute promyelocytic leukemia developed in a patient after receiving ICRF-159 treatment for cancer of the cecum. The patient had received adjuvant therapy with ICRF-159 for 2 years following surgery.

Animal bioassays

1. Rat long-term intraperitoneal (i.p.) injection studies: NCI, 1978. Groups of 35 male and 35 female Sprague-Dawley rats were injected i.p. with 48 or 96 mg/kg bw ICRF-159 three times per week for 52 weeks. All rats were then observed for 29-34 additional weeks without treatment before they were killed and examined for tumors. Untreated-control and vehicle-control groups each consisted of 10 rats/sex; pooled vehicle-control groups for each sex consisted of the 10 vehicle controls combined with 30 vehicle controls from similar bioassays. In female rats the incidence of uterine adenocarcinomas in the low- and high-dose groups, 10/33 and 11/32, respectively, was significantly increased above the incidence of 0/38 in pooled controls ($p<0.001$). The trend of increasing incidence with increasing dose was also significant ($p<0.001$). There was a high mortality rate among male rats given ICRF-159, and no significant increases in tumor incidence in groups of treated male rats were found. The NCI concluded that ICRF-159 is carcinogenic in female Sprague-Dawley rats.

2. Mouse long-term intraperitoneal injection studies: NCI, 1978. Groups of 35 male and 35 female $B6C3F_1$ mice were injected i.p. with 40 or 80 mg/kg bw ICRF-159 three times per week for 52 weeks. All mice were then observed for 29-34 additional weeks without treatment before they were killed and examined for tumors. Untreated-control and vehicle-control groups each consisted of 15 mice/sex; pooled-control groups consisted of the 15 vehicle controls of each sex combined with 30 vehicle controls of each sex from similar bioassays. In female mice, the incidence of hematopoietic neoplasms combined (histiocytic lymphomas, lymphocytic lymphomas or lymphocytic leukemias) in the low- (5/31) and high-dose groups (9/34) was significantly increased ($p = 0.038$, $p = 0.002$, respectively) above the incidence in pooled controls (1/45). The incidence of these tumors in the high-dose group was also significantly increased above the incidence in vehicle controls (0/15, $p=0.026$). In addition, the trend of increasing incidence with increasing dose was significant ($p = 0.021$).

No treatment-related tumors were observed in males. The NCI concluded that ICRF-159 is carcinogenic in female $B6C3F_1$ mice.

Other relevant data

ICRF-159 was not mutagenic in the Ames *Salmonella* assay (McCann *et al*., 1975; Albanese and Watkins, 1985) but was mutagenic in Chinese hamster V79A cells (Witiak *et al*., 1977) and in mouse lymphoma L5178Y cells (Edgar, 1985). It was also positive in the mouse bone marrow micronucleus assay (Albanese and Watkins, 1985). In the Chinese hamster metaphase assay, ICRF-159 induced abnormal chromosome condensation, increased structural chromosome aberrations and increased the number of polyploid cells (Albanese and Watkins, 1985).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** for ICRF-159 due the induction of uterine adenocarcinomas in female rats and hematopoietic neoplasms (lymphocytic leukemia and lymphoma) in female mice. The level of concern is supported by case reports of cutaneous squamous cell carcinomas and acute myeloid leukemia in patients receiving ICRF-159 as long-term treatment for psoriasis; hematopoietic neoplasms in patients receiving the drug as long-term treatment for colorectal cancers, and several cases of AML observed in a prospective trial study of patients on ICRF-159 treatment. The similarities in the types of malignancies observed in animals and humans given ICRF-159 support a high level of carcinogenicity concern for the drug. This is further reinforced by positive findings in eukaryotic mutagenicity assays.

There is **NO IDENTIFIED CONCERN over the level of exposure** to ICRF-159 because it is not currently used as a therapeutic drug in the U.S. and has no other identified use.

References

Albanese R, Watkins PA (1985). The mutagenic activity of razoxane (ICRF 159): an anticancer agent. Br J Cancer **52**:725-31.

Bhavnani M, Wolstenholme RJ (1987). Razoxane and acute promyelocytic leukemia [letter]. *Lancet* **2**(8567):1085.

Edgar DH (1985). The mutagenic potency of 4 agents at the thymidine kinase locus in mouse lymphoma L5178Y cells *in vitro*: effects of exposure time. *Mutat Res* **157**(2-3):199-204.

Gilbert JM, Hellmann K, Evans M, Cassell PG, Taylor RH, Stoodley B, Ellis H, Wastell C (1986). Randomized trial of oral adjuvant razoxane (ICRF 159) in resectable colorectal cancer: five-year follow-up. *Br J Surg* **73**:446-50.

Horton JJ, Caffrey EA, Clark KGA, MacDonald DM, Wells RS, Daker MG (1984). Leukaemia in psoriatic patients treated with razoxane. *Br J Dermatol* **110**:633-34.

Horton JJ, MacDonald DM, Wells RS (1983). Epitheliomas in patients receiving razoxane therapy for psoriasis. *Br J Dermatol* **109**:675-78.

Joshi R, Smith B, Phillips RH, Barrett AJ (1981). Acute myelomonocytic leukaemia after razoxane therapy [letter]. *Lancet* **2**(8259):1343-44.

McCann JE, Choi E, Yamasaki E, Ames BN (1975). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. *Proc Natl Acad Sci* (U.S.A.) **72**:5135-39.

National Cancer Institute (NCI, 1978). Bioassay of ICRF-159 for possible carcinogenicity. CAS No. 21416-87-5. NCI-CG-TR-78. Bethesda, MD.

Witiak DT, Lee HJ, Hart RW, Gibson RE (1977). Study of transcyclopropylbis(diketopiperazine) and chelating agents related to ICRF 159. Cytotoxicity, mutagenicity and effects on scheduled and unscheduled DNA synthesis. *J Med Chem* **20**:630-35.

CARCINOGENICITY DATA SUMMARY: 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE

3'-Methyl-4-dimethylaminoazobenzene (MDAB, N,N-dimethyl-*p*-(*m*-tolylazo)-aniline; CAS No. 55-80-1) has been used as a model carcinogen in research laboratories, but is not known to occur naturally. It is not produced commercially in the U.S. (HSDB, 1997).

Carcinogenicity Data available:

Epidemiological studies

No reports of studies of the effects of long-term human exposure to MDAB were identified in a search of the scientific literature by OEHHA.

Animal bioassays

More than 100 positive carcinogenesis experiments have been reported (PHS 149, 1972-1992; RTECS, 1997; HSDB, 1997). Many of these were experimental or mechanistic studies in which the protocol differed from that expected in a standard bioassay. Administered by the oral route, MDAB has been shown to increase the incidence of liver cancer (hepatocellular carcinoma, cholangiocarcinoma) and other tumors in both sexes of rats and mice. Other studies used other routes, principally intraperitoneal or subcutaneous injections. Incidence rates in some studies apparently reached 80-100%. The chemical is widely used by researchers to study the induction and progression of liver tumors in rats. Though the chemical has also been tested in hamsters, guinea pigs and monkeys, the evidence of carcinogenicity in these species is equivocal, although this may in some instances be related to the design or duration of the studies. As examples, some of the more recent oral studies are summarized. (P values, where given, are calculated relative to the control group using Fisher's exact test.)

1. Mouse 12-week feeding studies: Aruna and Sivaramakrishnan, 1992, summarized in PHS 149, 1991-92. Fifteen male Swiss mice received 0.05% MDAB in the diet for 12 weeks. After 38 weeks, out of 11 survivors, 9 (82%, $p<0.01$) were found to have squamous cell carcinomas (SCC) of the stomach. Among 15 mice receiving control diet no stomach lesions were detected. In addition to the group receiving MDAB only, nine similar groups received a prior dietary supplementation with cumin or poppy seeds, basil leaves or similar plant materials. Some groups showed relative inhibition of the carcinogenic effect of MDAB, but in all treated groups incidence rates ranging from 23% to 80% for SCC of the stomach were observed.

2. Rat 10-week feeding study: Karaki *et al.*, 1991, summarized in PHS 149, 1991-92. Fifty male Donryu rats received diet containing 0.06% MDAB for 10 weeks, followed by plain diet for 10 further weeks. At sacrifice (20 weeks after start of dosing); 29/50 rats had liver tumors, 18 had cholangiofibrosis, and 3 showed oval cell proliferation. No control group was reported, but clearly this result differs from the expectation for similarly aged unexposed animals (< 45 weeks of age).

3. Rat 4 and 7-week feeding studies: Ohkawa *et al.*, 1991, summarized in PHS 149, 1991-92. Twenty-five male Donryu rats received diet containing 0.06% MDAB for 7 weeks, followed by plain diet. The total duration of the experiment was 25 weeks. At terminal sacrifice, 20/25 (80%, $p<<0.01$) rats had liver tumors (hepatocellular carcinomas and cholangiocarcinomas). A second group receiving the MDAB-containing diet for only 4 weeks had a liver tumor incidence of 3/25 (12%, $p<0.01$ relative to combined control groups). Three additional treated groups exposed similarly to MDAB also received i.p. injections of between 6.25 and 25 mg retinol acetate 5 days per week for 5 weeks. The treatments with retinol acetate increased the incidence of liver tumors to 95.8 - 100% in rats receiving MDAB for 7 weeks and to 16-22% in rats receiving MDAB for 4 weeks. Among four groups of 25 rats each receiving control diet, with or without retinol acetate injections, no liver tumors were observed.

Other relevant data

MDAB is genotoxic in several short-term tests. It was positive in the transformation of Fischer rat embryo cells, and it induced unscheduled DNA synthesis in rat primary hepatocytes, and sister-chromatid exchange in rat hepatoma

and rat esophageal tumor cell lines (RTECS, 1997). The chemical was also mutagenic in *Salmonella typhimurium* reverse mutation tests (TA 100 and TA 98), with metabolic activation. Binding to DNA has been reported (HSDB, 1997). Several structurally related aromatic amino compounds are identified by IARC (and under Proposition 65) as animal, and in some cases human, carcinogens.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** over MDAB since carcinogenicity has been reported in liver and at other sites in both sexes of rats, mice and possibly other species, by multiple routes. Tumor induction was noted with high incidences (in excess of 80%) and after relatively brief exposures (less than 4 weeks) in some experiments. The concern is reinforced by evidence of genotoxicity in studies *in vitro*, and structural similarities to other known carcinogens.

There is **NO IDENTIFIED CONCERN over the extent of exposure** to MDAB. This research chemical has not been reported to occur in the general environment, and appears not to be commercially produced in, or imported into, the U.S.

References

Hazardous Substances Data Bank (HSDB, 1997). National Library of Medicine. Bethesda, MD.

Public Health Service (PHS 149, 1972-1992). U.S. Department of Health, Education and Welfare/ Department of Health and Human Services, National Cancer Institute, Bethesda, MD.

Registry of Toxic Effects of Chemical Substances (RTECS, 1997). Database produced by the U.S. National Institute for Occupational Safety and Health. Version date 1/97: RTECS number BX8250000.

CARCINOGENICITY DATA SUMMARY: N-NITROSOMETHYL-N-HEPTYLAMINE

N-Nitrosomethyl-n-heptylamine (CAS No. 16338-99-1) is a nitrosamine and has been used by researchers to study the induction and progression of lung tumors in test animals.

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Hamster 40-week gavage studies: Lijinsky and Kovatch, 1988. Groups of 12 male and 12 female Syrian golden hamsters were administered N-nitrosomethyl-n-heptylamine by gavage. The animals were treated once a week for 40 weeks with a weekly dose of 6.8 mg (total dose of 2.1 mmole). Animals were allowed to live until natural death, or killed when moribund. Most of the exposed male hamsters developed tumors (83% developed liver tumors, 100% developed lung tumors, 67% developed forestomach tumors, and 67% developed tumors of the nasal mucosa). Similar incidence rates of tumors were also observed in the exposed female hamsters (82% developed liver tumors, 91% developed lung tumors, 64% developed forestomach tumors, and 45% developed nasal tumors). None of these tumors were observed in the male and female controls.

2. Hamster 35-week gavage study: Rehm and Lijinsky, 1994. Thirty-six male Syrian hamsters were administered with N-nitrosomethyl-n-heptylamine by gavage. The animals were treated once a week for 35 weeks with a weekly dose of 6.8 mg per animal. Ten male Syrian golden hamsters were used as controls. Twenty-two adenosquamous carcinomas and two squamous cell carcinomas of the lung were diagnosed in 20 treated hamsters at experimental weeks 35-46. Among the controls, no tumors were detected at week 46.

3. Hamster 35-week gavage study: Rehm and Devor, 1993. Twelve male Syrian golden hamsters were administered 6.8 mg of N-nitrosomethyl-n-heptylamine by gavage once a week for 35 weeks. Ten exposed hamsters developed bronchiolar tumors including lepidic bronchioalveolar carcinomas, acinar adenocarcinomas, and adeno-squamous and squamous-cell carcinomas.

4. Rat 34-week drinking water study: Lijinsky *et al*., 1983. Twenty male F344 rats were administered N-nitrosomethyl-n-heptylamine in drinking water at 140 mg/L for 34 weeks. Test animals were exposed to the chemical 5 days a week; on the other 2 days tap water was given to the animals. Nineteen rats survived at week 35 and none of the exposed animals survived beyond week 55. Carcinomas of the esophagus (15/20), the liver (6/20), the lungs (11/20), and the nasal cavity (2/20) were observed in the exposed male rats. There was no control group.

5. Rat 25-week gavage study: Lijinsky *et al*., 1983. Two groups of 20 male F344 rats were administered either 7 or 17.2 mg N-nitrosomethyl-n-heptylamine by gavage, twice a week. Only 2 of the high-dose animals survived to week 35, 4 of the low-dose animals survived to week 60. Incidence rates of liver carcinoma for the low- and high-dose groups were 20/20 and 10/20, respectively. Incidence rates of lung adenomas and carcinomas for the low-dose group were 20/20 and 2/20, and for the high-dose groups 7/20 and 2/20, respectively. There was no control group.

6. Rat subcutaneous injection study: Druckrey *et al*., 1967. N-Nitrosomethyl-n-heptylamine was administered to 20 BD rats via subcutaneous injection (40 or 80 mg/kg), once a week for 58 weeks. Most test animals died after 12 months; one lung tumor was observed in 1 rat.

Other Relevant Data:

N-Nitrosomethyl-n-heptylamine has been shown to be mutagenic in *S. typhimurium* strain TA1535 with metabolic activation (Andrews and Lijinsky, 1980). N-Nitrosomethyl-n-heptylamine is a nitrosamine, a group of chemicals that are known for their mutagenicity and carcinogenicity.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** as N-nitrosomethyl-n-heptylamine has been shown to induce tumors of the lungs, liver, forestomach, and nasal mucosa in male and female hamsters, in spite of the short exposure durations. The level of concern is further supported by the observation of carcinomas of the esophagus, liver, and lungs in male rats treated with N-nitrosomethyl-n-heptylamine. Additional support is provided by positive mutagenicity results, and structure-activity relationships.

There is **NO IDENTIFIED CONCERN over the extent of exposure** of N-nitrosomethyl-n-heptylamine, as it is used solely as a model carcinogen for research purposes, and is not known to occur naturally.

References

Andrews AW, Lijinsky W (1980). The mutagenicity of 45 nitrosamines in *Salmonella typhimurium*. *Teratogenesis, Carcinogenesis, and Mutagenesis* **1**:295-303.

Druckrey H, Preussmann R, Ivankovic S, Schmähl D (1967). Organotrope carcinogene wirkungen bei 65 verschiedenen N-nitroso-verbindungen an BD-ratten. *Zeitschrift für Krebsforschung* **69**:103-201.

Lijinsky W, Reuber MD, Singer GM (1983). Induction of tumors of the esophagus in rats by nitrosomethylalkylamines. *J Cancer Res Clin Oncol* **106**:171-175.

Lijinsky W, Kovatch RM (1988). Comparative carcinogenesis by nitrosomethylalkylamines in Syrian Hamsters. *Cancer Research* **48**:6648-6652.

Rehm S, Devor DE (1993). Acute effects of 4-ipomeanol on experimental lung tumors with bronchiolar or alveolar cell features in Syrian hamsters or C3H/HeNCr mice. *J Cancer Res Clin Oncol* **120**:41-50.

Rehm S, Lijinsky W (1994). Squamous metaplasia of bronchiolar cell-derived adenocarcinoma induced by N-nitrosomethyl-n-heptylamine in Syrian hamsters. *Vet Pathol* **31**:561-571.

CARCINOGENICITY DATA SUMMARY: N-NITROSO-N-PENTYLUREA

N-Nitroso-n-pentylurea (N-amyl-n-nitrosourea; CAS No. 10589-74-9) is used by researchers to study the induction and progression of tumors in test animals.

Carcinogenicity Data available:

Epidemiological studies

No data on the carcinogenic effects of this chemical in humans were identified.

Animal bioassays

1. Rat 60-week drinking water studies: Fujii *et al*., 1980. Groups of 35 male and 33 or 35 female Donryu rats were administered N-nitroso-n-pentylurea in drinking water at 100, 200, or 400 ppm, 5 days a week for 60 weeks. Fourteen male and 20 female rats were used as controls. Tumors of the digestive tract was detected in most animals (63 to 83%) exposed to N-nitroso-n-pentylurea but none in the controls. Incidence rates of carcinomas of the forestomach in the controls, low-, mid-, and high-dose animals were 0/14, 6/35, 2/34, and 8/35 for male rats and 0/20, 4/35, 1/33, and 2/33 for female rats. Incidence rates of carcinomas of the esophagus for the controls, low-, mid-, and high-dose animals were 0/14, 0/35, 3/34, and 4/35 for male rats and 0/20, 1/35, 3/33, and 3/33 for female rats, respectively. There were dose-related increases in hematopoietic cancer in both male and female rats. Incidence rates for the controls, low-, mid-, and high-dose animals were 0/14, 1/35, 6/34, and 7/35 for male rats and 0/20, 1/35, 2/33, and 6/33 for female rats, respectively. Statistically significant increases of hepatocellular adenomas were also detected in the females (0/20, 0/35, 5/33, and 8/33 for the controls, low-, mid-, and high-dose animals, respectively). Tumors of the mammary gland were found in females in all dosed groups, however, the incidence rates were not statistically higher than that of the controls.

2. Rat 52-week drinking water study: Hirose *et al*., 1979. Fifty-four female Donryu rats were continuously given 15-20 ml/rat/day of a 400 ppm solution of N-nitroso-n-pentylurea in drinking water until they died or became moribund and were killed. The last rat died on day 385 of the experiment. Fourteen rats died before week 24, the time when the first tumor appeared, and were excluded from the data evaluation. Tumors were found in all the rats (40/40) exposed to N-nitroso-n-pentylurea: carcinomas of the oral cavity and pharynx (35/40), carcinomas of the esophagus (36/40), and carcinomas of the forestomach (8/40). There was no control group.

3. Rat 50-week gavage studies: Lijinsky and Kovatch, 1989. Groups of 12 male and 12 female F344 rats were administered N-nitroso-n-pentylurea by gavage twice a week for 50 weeks. The total dose administered was 2 mmoles for both sexes. Same number of male and female rats were maintained as controls. The authors. reported that N-nitroso-n-pentylurea induced forestomach tumors (12/12), thyroid tumors (3/12), lung tumors (8/12), mesotheliomas (6/12), and skin tumors (4/12) in the exposed male rats. The compound also induced forestomach tumors (10/12), thyroid tumors (2/12), lung tumors (7/12), tumors of the nervous system (2/12), mammary gland tumors (3/12), and tumors of the uterus (8/12) in the exposed female rats. No tumors were found in the control males and 1 thyroid tumor, 1 lung tumor, and 2 tumors of the uterus were observed in the control females.

4. Rat single subcutaneous injection study: Zeller *et al*., 1982. Groups of 24 or 25 BD IX rats of various ages were administered with a single subcutaneous injection of N-nitroso-n-pentylurea with a dose equal to 10% of the LD_{50} (in adult rats). Incidence rates of neurogenic tumors in rats exposed at 1 day after birth, 10 days after birth, 30 days after birth, and 60 days after birth were 12/25, 13/24, 15/25, and 4/25, respectively. Tumors identified included brain tumors, tumor of the cranial nerves, peripheral nervous system tumors, and neurogenic tumors of the heart.

5. Rat single subcutaneous or peroral injection study: Zeller *et al*., 1982. Twenty-four 3-month-old BD IX rats were administered a single peroral injection of N-nitroso-n-pentylurea with a dose equal to 570-600 mg/kg. Tumors observed included heart (6/24), gastrointestinal tract (7/24), brain (1/24), kidney (1/24), leukemia (2/24), and subcutis (2/24). Thirty-seven 3-month-old BD IX rats were administered with a single subcutaneous

injection of N-nitroso-n-pentylurea with a dose equal to 510-900 mg/kg. Tumors observed included heart (3/37), gastrointestinal tract (2/37), brain (2/37), and subcutis (18/37).

6. Mouse 50-week skin painting study: Lijinsky and Winter, 1981. Twice a week, one 25 µL drop of 0.04M N-nitroso-n-pentylurea in acetone was applied to the shaved interscapular skin of 20 female Swiss mice. Twenty mice received acetone alone and were used as controls. The treatment continued for 50 weeks, after which the animals were observed until death or 100 weeks. The first skin tumor in treated mice appeared at week 35 and by week 95, 11 of the 20 mice exposed to N-nitroso-n-pentylurea developed skin tumors. No skin tumors were observed in the control group at week 95.

7. Mouse 50-week skin painting study: Lijinsky and Reuber, 1988. Twice a week, one 25 µL drop of 0.04M N-nitroso-n-pentylurea in acetone was applied to the shaved interscapular skin of 20 female Swiss mice. Twenty mice received acetone alone and were used as controls. The treatment lasted for 50 weeks, after which the animals were observed until death or 110 weeks. Increased incidences of tumors of the forestomach, tongue, and oropharnyx (6/20 exposed, 0/20 controls), tumors of the mammary gland (3/20 exposed, 0/20 controls), and tumors of the ovary (5/20 exposed, 0/20 controls) were reported.

Other Relevant Data:

N-Nitroso-n-pentylurea has been shown to be mutagenic in a test strain of *E. coli* without metabolic activation (Kawazoe *et al*., 1986). It was also mutagenic in *S. typhimurium* TA1535 with and without metabolic activation (Andrews and Lijinsky, 1984) and positive in the prophage induction assay (Lijinsky *et al*., 1987). N-Nitroso-n-pentylurea has also been shown to induce chromosomal aberrations in Chinese hamster cells *in vitro* (Ishidate and Odashima, 1977). N-Nitroso-n-pentylurea is a nitrosamine, a group of chemicals that are known for their mutagenicity and carcinogenicity.

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** as N-nitroso-n-pentylurea has been shown to induce tumors of the digestive tract, lung, and hematopoietic systems in male and female rats. It also induced hepatocellular adenomas and tumors of the uterus in female rats. The level of concern is supported by reports showing that when N-nitroso-n-pentylurea was painted onto the back of female mice, it induced skin tumors, tumors of the forestomach, tongue, and oropharnyx, tumors of the mammary gland, and tumors of the ovary. Additional support is provided by positive mutagenicity results and structure-activity relationships.

There is **NO IDENTIFIED CONCERN over the extent of exposure** of N-nitroso-n-pentylurea as it is used solely as a model carcinogen for research purposes, and is not known to occur naturally.

References

Andrews AW, Lijinsky W (1984). N-Nitrosamine mutagenicity using the *Salmonella*/Mammalian-microsome mutagenicity assay in "*Genotoxicology of N-nitroso compounds*". Ed. Rao TK, Lijinsky W, Epler JL. Topics in chemical mutagenesis, Vol. 1. Series Ed. Serres FJ. Plenum Press, New York, NY.

Fujii K, Nakadate M, Ogiu T, Odashma S (1980). Induction of digestive tract tumors and leukemias in Donryu rats by administration of 1-amyl-1-nitrosourea in drinking water. *Gann* **71**:464-470.

Hirose M, Maekawa A, Kamiya S, Odashima S (1979). Carcinogenic effect of N-ethyl- and N-amyl-N-nitrosourethans on female Donryu rats. *Gann* **70**:653-662.

Kawazoe Y, Fujiura T, Kohda K (1986). Studies on chemical carcinogens and mutagens. XXXVII. A simple method to predict ultimate structures of chemical mutagens, and probably carcinogens. *Chem Pharm Bull* **34**:1755-1763.

Ishidate M, Odashima S (1977). Chromosome tests with 134 compounds on Chinese hamster cells *in vitro* - a screening for chemical carcinogens. *Mutat Res* **48**:337-354.

Lijinsky W, Winter C (1981). Skin tumors induced by painting nitrosoalkylureas on mouse skin. *J Cancer Res Clin Oncol* **102**:13-20.

Lijinsky W, Elespuru RK, Andrews AW (1987). Relative mutagenic and prophage-inducing effects of mono- and di-alkyl nitrosoureas. *Mutat Res* **178**:157-165.

Lijinsky W, Reuber MD (1988). Neoplasms of the skin and other organs observed in Swiss mice treated with nitrosoalkylureas. *J Cancer Res Clin Oncol* **114**:245-249.

Lijinsky W, Kovatch RM (1989). Similar carcinogenic actions of nitrosoalkylureas of varying structure given to rats by gavage. *Toxicol Ind Health* **5**(6):925-935.

Zeller WJ, Ivankovic S, Habs M, Schmähl D (1982). Experimental chemical production of brain tumors. In: *Brain tumors in the chemical industry*. Ed. Selikoff IJ, Hammond EC. *Ann NY Acad Sci*. **381**. New York, NY.

CARCINOGENICITY DATA SUMMARY: N-(2-METHOXYETHYL)-N-NITROSOUREA

N-(2-methoxyethyl)-N-nitrosourea (1-nitrosomethoxyethylurea, 1-(2-methoxyethyl)-1-nitrosourea, nitroso-2-methoxyethyl urea, M-HEU; CAS No. 108278-70-2) is a monoalkylnitrosourea. This compound has been used as a laboratory research chemical. The potential formation of this compound in the bladders of individuals with bacterial bladder infections as a result of the interaction between urinary amides and bacterially produced nitrite has been suggested (Lijinsky *et al*., 1992).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to N-(2-methoxyethyl)-N-nitrosourea were found in the literature.

Animal bioassays

1. Rat 30-week gavage studies: Lijinsky and Kovatch, 1988. Groups of 12 F344 rats/sex/dose were administered 0.2 mL N-(2-methoxyethyl)-N-nitrosourea in an ethyl acetate/corn oil (1:2) solution at doses of 8 mg/mL and 16 mg/ml via gavage, twice weekly for 30 weeks (24 weeks for high-dose females; 28 weeks for high-dose males). Groups of 20 rats/sex served as vehicle controls, and were administered 0.2 ml of an ethyl acetate/corn oil (1:2) solution for 60 weeks. Increased incidences of tumors were observed at multiple sites in treated animals of both sexes. In treated males, tumors of the thymus, lung, forestomach, duodenum, colon, tongue, and brain were observed. In treated females, tumors of the thymus, mammary gland, lung, tongue, forestomach, duodenum, colon, and Zymbal gland were observed.

2. Rat 17-week intravesicular injection study: Lijinksy *et al*., 1992. A group of 12 female F344 rats received injections of 6.4 mg N-(2-methoxyethyl)-N-nitrosourea in a 25% ethanol solution directly into the bladder (intravesicularly), twice per week for 17 weeks. Twelve females served as controls, receiving 0.2 ml 25% aqueous ethanol twice per week for 30 weeks. An increased incidence of malignant tumors of the bladder was observed in treated animals (9/10) as compared with controls (1/12). Several types of bladder tumors were observed in the treated animals, including 5 transitional cell tumors, 3 squamous cell tumors, 4 fibrosarcomas, and one leiomyoma.

Other Relevant Data

N-(2-methoxyethyl)-N-nitrosourea is a direct-acting alkylating agent, and a powerful direct-acting bacterial mutagen, inducing mutations in *Salmonella* (Lijinsky *et al*., 1987) and *E. coli* (Kohda *et al*., 1987). N-(2-methoxyethyl)-N-nitrosourea was positive in the *E. coli* prophage inducing assay, where prophage induction is one manifestation of the SOS response to chromosomal damage in *E. coli* (Lijinsky *et al*., 1987). Several other monoalkylnitrosoureas have been shown to induce bladder tumors when injected intravesicularly into female rats (Lijinsky *et al*., 1992).

Preliminary evaluation of carcinogenicity and exposure data:

There is a **HIGH** level of **carcinogenicity concern** since oral administration of N-(2-methoxyethyl)-N-nitrosourea induced tumors at multiple sites in male and female rats after a relatively short exposure period of 30 weeks and since direct instillation into the bladder (modeling a relevant route of human exposure) over a period of 17 weeks induced bladder tumors in female rats. The level of concern is supported by positive evidence of genotoxicity from bacterial tests and chemical structural analogies with other monoalkylnitrosoureas with demonstrated tumorigenic activity in female rats.

There are **INADEQUATE DATA** to assign a level of **concern over the extent of exposure** to N-(2-methoxyethyl)-N-nitrosourea since it is not known whether endogenous formation of this compound occurs, or whether the compound is present in any environment other than the research laboratory.

References

Kohda KH, Ninomiya S-I, Washizu K, Shiraki K, Ebie M, Kawazoe Y (1987). Mutagenicity of a series of N-alkyl-, N-hydroxyalkyl-, N-haloalkyl- and N-carboxyalkyl-N-nitrosoureas in *Escherichia coli* tester strains: Dependence on the *uvrA* DNA-repair system. *Mutat Res* **177**:219-228.

Lijinsky W, Elespuru RK, Andrews AW (1987). Relative mutagenic and prophage-inducing effects of mono- and di-alkyl nitrosoureas. *Mutat Res* **178**:157-165.

Lijinsky W, Kovatch BJ (1988). Carcinogenesis by nitrosohydroxyethylurea and nitrosomethoxyethylurea in F344 rats. *Jpn J Cancer Res* **79**:181-186.

Lijinsky W, Thomas BJ, Kovatch RM (1992). Systemic and local carcinogenesis by directly acting *N*-nitroso compounds given to rats by intravesicular administration. *Carcinogenesis* **13**:1101-1105.

CARCINOGENICITY DATA SUMMARY: C.I. ACID BLUE 9 AND ITS SALTS

C.I. Acid Blue 9 (FD&C Blue No.1, Brilliant Blue FCF; CAS No. 2650-18-2) is a water-soluble triphenylmethane color additive that has been in continuous use in foods, drugs and cosmetics since the late 1800s. (The chemical name is ammonium, ethyl (4-p-(ethyl(m-sulfbenzyl)amino)-alpha-(o-sulfophenyl)benzylidene)2,5-cyclohexadien-1-ylidene)(m-sulfobenzyl)-, hydroxide, inner salt). C.I. Acid Blue 9 is one of nine synthetic color additives permitted for direct addition to human food in the U.S. (FDA, 1993). The disodium salt of this dye is used in foods while the diammonium salt of the dye has had limited use in drugs and cosmetics. Common food uses include beverages, dairy product powders, jellies, confections, condiments, icings, syrups and extracts (FDA, 1993). This color additive does not appear on the FDA list for chemicals generally regarded as safe (GRAS). IARC (1987) categorizes this agent as having limited evidence for carcinogenicity in experimental animals, no data in humans (Group 3). This evaluation was made only on the basis of the data considered earlier (IARC, 1978), which have subsequently been extended by additional bioassay and genotoxicity studies.

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to C.I. Acid Blue 9 were found in earlier searches by IARC (1978, 1987) or recently by OEHHA.

Animal bioassays

1. Rat *in utero* and lifetime feeding studies: Borzelleca *et al.*, 1990. Male and female Charles River CD rats (60/sex/group) were fed diets containing 0, 0.1, 1.0, or 2.0% C.I. Acid Blue 9 for approximately 2 months after mating ("*in utero* phase"). Two rats of each sex from each litter were randomly selected for continued dosing ("chronic phase"), for a total of 70 rats/sex/dose. Both male and female rats were fed diets containing 0, 0.1, 1.0, or 2.0% C.I. Acid Blue 9 throughout the chronic dosing phase. These doses corresponded to an average daily compound consumption of 0, 50, 514, or 1072 mg/kg-day in males and 0, 62, 631, or 1319 mg/kg-day in females. Dosing was continued for 116 weeks for males and 111 weeks for females. No treatment-related increase in tumors was observed.

2. Mouse 104-week feeding studies: Borzelleca *et al.*, 1990. Male and female Charles River CD-1 mice (60/sex/group) were fed diets containing 0, 0.5, 1.5 or 5.0% C.I. Acid Blue 9, corresponding to an average daily compound consumption of 0, 661, 2064, or 7354 mg/kg/day (males) and 0, 819, 2562, or 8966 mg/kg/day (females), for 104 weeks. An increased incidence of hemangiomas of the spleen in females was statistically significant only by an unadjusted trend analysis. The authors concluded the hemangiomas were spontaneous and not related to exposure to C.I. Acid Blue 9. No other treatment-related increases in tumors were observed.

3. Mouse 80-week feeding studies: Rowland *et al.*, 1977[1], as reported by IARC, 1978. Groups of 48 male and 50 female ASH/CSI mice were fed diets containing 0, 0.015, 0.15 or 1.5% C.I. Acid Blue 9 (disodium salt) for 80 weeks, corresponding to estimated doses of 0, 20, 200 or 2000 mg/kg/day. Seven kidney tumors (6 adenomas and 1 adenocarcinoma) were observed in the mid-dose males (7/30 versus 1/44 in controls) ($p<0.05$). However, there was no indication of a dose-related effect, as an increase of kidney tumors was not observed for the rats in the low- or high-dose groups.

4. Rat 75-week feeding studies: Mannell *et al.*, 1962. Male and female Wistar rats (15/sex/group) were fed diets containing 0, 0.03, 0.3, or 3% C.I. Acid Blue 9 (disodium salt) for 75 weeks. No significant increase in tumor incidence was observed in treated mice compared with controls. However, IARC (1978) concluded that histological examination of only 5 rats/sex/group, as was done in this study, was inadequate for determination of carcinogenic activity.

---

[1] This study is reported in IARC (1978) as "in press"; however, the paper was never published.

5. Rat 2-year feeding studies: Hansen *et al.*, 1966. Male and female Osborne-Mendel rats (12/sex/group) were fed diets containing 0, 0.5, 1.0, 2.0, or 5.0% C.I. Acid Blue 9 (disodium salt) for 2 years. At the end of the study, 225 of 240 rats were examined. No treatment-related increases in tumors were observed compared to controls.

6. Rat long-term feeding study: Klinke, unpublished, as reported by PHS 149, 1969. 85 rats (strain, type or sex not recorded) were fed the dye as 0.1% of their food (10-15 mg total dose). No tumors were observed.

7. Rat 2-year feeding studies: Willheim and Ivy 1953, as reported by PHS 149, 1957. Ten rats (male and female mixed) were fed diets containing 4% C.I. Acid Blue 9 for 2 years. No tumors were observed.

8. Dog 1-year feeding studies: Hansen *et al.*, 1966. Beagle dogs, 6-7 months of age, were fed C.I. Acid Blue 9 in their diets at concentrations of 0% (1 male, 1 female), 1.0% (2 males, 2 females) and 2.0% (4 males, 2 females) for 1 year. No tumors were observed. The short study duration and small numbers of animals make the utility of this study very limited.

9. Rat 2-year s.c. injection studies: Hansen *et al.,* 1966. Male and female Osborne-Mendel rats (9/sex) received weekly subcutaneous injections of 30 mg C.I. Acid Blue 9 disodium salt as a 3% aqueous solution for 2 years. Nearly all rats (16/18) developed injection-site sarcomas while 18 saline-injected controls remained tumor-free. Borzelleca *et al.* (1990) concluded that the injection-site sarcomas produced in this and other subcutaneous injection studies were the result of an alteration in the surface activity of the subcutaneous tissue by the repeated injection of highly concentrated C.I. Acid Blue 9 solutions.

10. Rat long-term s.c. injection studies: Gross, 1961, as reported by IARC, 1978. Rats (sex and strain unspecified) receiving twice weekly subcutaneous injections of 7.4 or 20 mg C.I. Acid Blue 9 (37% pure) for an average period of 20.5 months developed injection-site fibrosarcomas that were dose-dependent (10/48 and 20/27 for low- and high-dose rats, respectively). No injection-site sarcomas were found in vehicle controls. When the study was repeated using C.I. Acid Blue 9 that was 90% pure, incidence of injection-site fibrosarcomas was observed to be 18/25 in the low-dose group and 25/40 in the high-dose group. The treatment periods were 17 and 12.5 months for the low- and high- dose groups, respectively.

11. Rat 45-week s.c. injection studies: Mannell and Grice, 1964. Male and female Wistar rats (10/sex) receiving weekly subcutaneous injections of a 4% solution of C.I. Acid Blue 9 disodium salt (0.5 ml) for 45 weeks and terminated at 71 weeks did not develop tumors.

12. Rat long-term s.c. injection studies: Nelson and Hagan, 1953. Osborne-Mendel rats (18/sex) were given weekly s.c. injections of 1mL of "2 or 3%" Brilliant Blue FCF (C.I. Acid Blue 9) for 94-99 weeks. No tumor (fibrosarcoma) incidence data was provided. The authors stated: "After 40-45 weeks tumors appeared with . . . Brilliant Blue FCF." No further information on tumor site was provided. This paper appears to be an interim report presented as part of a meeting proceedings.

Other relevant data

C.I. Acid Blue 9 was not mutagenic to several strains of *Salmonella typhimurium* tested with or without S9 (Longstaff *et al.*, 1984; Ishidate *et al.*, 1984; and 6 additional studies reviewed by Borzelleca *et al.*, 1990). However, in a more recent study, C.I. Acid Blue 9 was mutagenic in the *Salmonella* assay and the mouse lymphoma TK+/- assay (Cameron *et al.*, 1987). An *in vivo - in vitro* unscheduled DNA synthesis assay indicated that C.I. Acid Blue 9 was mutagenic (Kornbrust and Barfknecht, 1985). C.I. Acid Blue 9 was not mutagenic by the DNA repair with "rec-Assay" (Haveland-Smith and Combes, 1980) or the *in vivo* mouse micronucleus assay (Hayashi *et al.*, 1988). However, C.I. Acid Blue 9 did produce chromosomal aberrations *in vitro* in CHL cells (Ishidate *et al.*, 1984). C.I. Acid Blue 9 was negative in the Fischer rat embryo cell transformation (Price *et al.*, 1978) and BHK21 cell transformation assays (Longstaff *et al.*, 1984).

Preliminary evaluation of carcinogenicity and exposure data:

C.I. Acid Blue 9 has not been placed on the candidate list. There is negative or equivocal evidence of carcinogenicity by the oral route in numerous rat and mouse feeding studies. IARC (1978) concluded that C.I. Acid Blue 9 is carcinogenic by the subcutaneous route (an unlikely route of exposure for humans), and all tumors appear to be at the site of injection. The findings in short-term mutagenicity assays are mixed, and do not add to the level of concern.

There is a **HIGH** level of **concern over the extent of exposure,** because of the common use of this color additive in food products.

References

Borzelleca JF, Depukat K, Hallagan JB (1990). Lifetime toxicity/carcinogenicity studies of FD & C Blue No. 1 (brilliant blue FCF) in rats and mice. *Food Chem Toxicol* **28**(4):221-234.

Cameron TP, Hughes TJ, Kirby PE, Fung VA, Dunkel VC (1987). Mutagenic activity of 27 dyes and related chemicals in the *Salmonella*/microsome and mouse lymphoma TK+/- assays. *Mutat Res* **189**:223-261.

Food and Drug Administration (FDA, 1993). Food color facts, FDA/IFIC brochure: January 1993 (6 pages). FDA World Wide Web site at URL http\\www.fda.gov, May 1998.

Gross E (1961). Über die Erzeugung von Sarkomen durch die besonders gereinigten Triphenylmethanfarbstoffe Lichtgrun SF und Patentblau AE bei der wiederholten subcutanen Injektion an der Ratte. *Z Krebsforsch* **64**:287-304.

Hansen WH, Fitzhugh OG, Nelson AA, Davis KJ (1966). Chronic toxicity of two food colors, brilliant blue FCF and indigotine. *Toxicol Appl Pharmacol* **8**:29-36.

Haveland-Smith RB, Combes RD (1980). Screening of food dyes for genotoxic activity. *Food Cosmet Toxicol* **18**:215-221.

Hayashi M, Kishi M, Sofuni T, Ishidate M Jr (1988). Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. *Food Chem Toxicol* **26**:487-500.

International Agency for Research on Cancer (IARC, 1978). Brilliant Blue FCF Diammonium and Disodium Salts. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Some Aromatic Amines and Related Nitro Compounds - Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals*. Volume 16 pp. 171-186. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of carcinogenic risks to humans, Overall evaluation of carcinogenicity: An updating of IARC monographs Volumes 1 to 42.* Supplement 7, p. 59. IARC, Lyon, France.

Ishidate M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A (1984). Primary mutagenicity screening of food additives currently used in Japan. *Food Chem Toxicol* **22**:623-636.

Kornbrust D, Barfknecht T (1985). Testing of 24 food, drug, cosmetic and fabric dyes in the *in vitro* and the *in vivo*/in vitro rat hepatocyte primary culture/DNA repair assays. *Environ. Mutagen* **7**:101-120.

Longstaff E, McGregor D, Harris W, Robertson J, Poole A (1984). A comparison of the predictive values of the *Salmonella*/microsome mutation and BHK21 cell transformation assays in relation to dyestuffs and similar materials. *Dyes Pigments* **5**:65-82.

Mannell WA, Grice HC (1964). Chronic toxicity of brilliant blue FCF, blue VRS, and green S in rats. *J Pharm Pharmacol* **16**:56-59.

Mannell WA, Grice HC, Allmark MG (1962). Chronic toxicity studies on food colours. V. Observations on the toxicity of brilliant blue FCF, guinea green B and benzyl violet 4B in rats. *J Pharm Pharmacol* **14**:378-384.

Nelson AA, Hagan EC (1953). Production of fibrosarcomas in rats at site of subcutaneous injection of various food dyes. *Fed Proc* **12**:397-398.

Public Health Service (PHS 149, 1957). Survey of compounds which have been tested for carcinogenic activity. Supplement 1. US Department of Health, Education, and Welfare, p. 32.

Public Health Service (PHS 149, 1969). Survey of compounds which have been tested for carcinogenic activity. Supplement 2. US Department of Health, Education, and Welfare, p. 113.

Price PJ, Suk WA, Freeman AE, Lane WT, Peters RL, Vernon ML, Heubner RJ (1978). *In vitro* and *in vivo* indications of the carcinogenicity and toxicity of food dyes. Int J Cancer **21**:361-367

CARCINOGENICITY DATA SUMMARY: C.I. ACID RED 51

C.I. Acid Red 51 (FD&C Red No. 3, erythrosine, erythrosine B, tetraiodofluorescein sodium salt; CAS No. 16423-68-0) is used as a dye in foods, drugs and cosmetics. In 1986, 252,000 pounds of C.I. acid red 51 were certified for use by the U.S. Food and Drug Administration (FDA) (Borzelleca *et al.*, 1987). In 1990, FDA discontinued the provisional listing of all water insoluble forms of C.I. acid red 51 and the water soluble forms used in external drugs and cosmetics (FDA, 1998).

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Rat *in utero* and lifetime feeding studies: Borzelleca *et al.*, 1987. Groups of 60 male and 60 female Charles River CD rats were fed diet containing 0, 0.1%, 0.5%, 1%, or 4% of C.I. acid red 51 for about two months before mating. A maximum of two rats of each sex from each litter were randomly selected for the long-term toxicity study. There were 70 F_1 males and 70 F_1 females in each dose group and they were exposed to the same diet as their parents for 122 to 129 weeks. The average intakes of C.I. acid red 51 were 49, 251, 507, and 2464 mg/kg-day by the male rats and 61, 307, 641, and 3029 mg/kg-day by the female rats fed the 0.1%, 0.5%, 1%, or 4% diets, respectively. Survival in all groups exceeded 50% at 24 months. There were statistically significant ($p<0.01$) increases in the incidence of thyroid follicular cell hyperplasia in males in the two highest dose groups and of thyroid follicular cell adenomas in males in the highest dose group. There was an increase in the incidence of thyroid follicular cell adenomas in females in the three highest dose groups, but the incidence rates were not statistically different from those of the controls. No increases in the incidence of neoplastic lesions were observed in other tissues for either males or females.

2. Rat 86-week feeding studies: Hansen *et al.*, 1973a. Groups of 25 male and 25 female Osborne-Mendel rats were exposed to C.I. acid red 51 in diet at 0.5, 1, 2, or 4% for 86 weeks. The controls consisted of 50 males and 50 females. At the end of the treatment period, the animals were fed the control diet until the experiment was terminated at 2 years. No statistically significant increases in tumors were observed in the exposed animals.

3. Rat 86-week gavage studies: Hansen *et al.*, 1973a. Groups of 25 male and 25 female Osborne-Mendel rats were administered C.I. acid red 51 by gavage at 0, 100, 235, 750, or 1500 mg/kg twice weekly for 86 weeks. At the end of the treatment period, the animals were maintained until the end of the study (2 years). No statistically significant increases in tumors were observed in the exposed animals.

4. Rat 2-year feeding studies: Hansen *et al.*, 1973b. Groups of 12 male and 12 female Osborne-Mendel rats were exposed to C.I. acid red 51 in diet at 0, 0.5, 1, 2, or 5% for 2 years. No statistically significant increases in tumors were observed in the exposed animals.

5. Rat lifetime subcutaneous injection study: Umeda, 1956. Twenty albino rats of a mixed strain (Saitama strain) were injected with C.I. acid red 51 once every week. Seven rats survived 300 days or more; the total administered dose ranged from 1100 to 2650 mg. No treatment-related tumors were observed in the exposed animals.

6. Mongolian gerbil 105-week feeding studies: Collins and Long, 1976. Groups of male and female gerbils were fed with a diet containing 1, 2 or 4% C.I. acid red 51 for 105 weeks. There were 7-13 animals/sex/group. Sixty-four gerbils of both sexes were fed unadulterated rat chow and were used as controls. A dose-related change in the thyroid reminiscent of human nodular goitre was observed in the gerbils exposed to C.I. acid red 51. No treatment-related tumors were observed in either males or females.

7. Mongolian gerbil 97-week gavage studies: Collins and Long, 1976. Groups of male and female gerbils were administered with 200, 750 or 900 mg/kg C.I. acid red 51 twice weekly by gavage for 97 weeks. There were 6-11 animals/sex/group. Sixty-three gerbils of both sexes were used as controls and were intubated with distilled water. No treatment-related tumors were observed in males or females.

Other Relevant Data:

C.I. acid red 51 was shown to be negative in the *Salmonella* assay (Cameron *et al*., 1987; Haveland-Smith *et al*., 1981; Lin and Brusick, 1986). Lück *et al*. (1963) reported that C.I. acid red 51 was shown to induce both forward and reverse mutations in *Escherichia coli*. Brown *et al.* (1978) showed that C.I. acid red 51 was negative for DNA repair in *Escherichia coli* and in fluctuation tests with *Salmonella typhimurium*. Lakdawalla and Netrawali (1988) reported that in the *Bacillus subtilis* multigene sporulation assay, C.I. acid red 51 under fluorescent light showed a strong mutagenic response with *B. subtilis* 168, but not with *B. subtilis* hcr-9. In the dark, the magnitude of mutagenicity of the dye was decreased.

Conflicting results were reported for the mouse lymphoma TK+/-assay. Cameron *et al*. (1987) showed that C.I. acid red 51 was positive in this assay both with and without metabolic activation. However, Lin and Brusick (1986) reported that C.I. acid red 51 was negative in the mouse lymphoma TK+/-assay. Rogers *et al.* (1988) demonstrated that C.I. acid red 51 was non-mutagenic to V79 cells at the HGPRT and Na+, K+ -ATPase gene loci, and did not increase the frequency of SCE with or without activation by rat hepatocytes. However, they showed that C.I. acid red 51 induced the formation of micronuclei in V79 cells, but only at the highest dose tested (300 µg/ml). Lin and Brusick (1986) administered C.I. acid red 51 to CD-1 mice by intravenous injection and reported that C.I. acid red 51 did not induce significant increases in micronucleus frequencies in bone marrow polychromatic erythrocytes in the mouse. Ishidate *et al.* (1984) reported that C.I. acid red 51 induced structural chromosomal aberrations in a Chinese hamster fibroblast cell line.

Jennings *et al.* (1990) fed C.I. acid red 51, sodium iodide, or fluorescein in diet to rats for three weeks and measured levels of thyroid releasing hormone (TRH) *in vivo*. The authors suggested that C.I. acid red 51 increases the pituitary thyroid stimulating hormone (TSH) response to TRH by altering thyrotroph cell conversion of T4 to T3. They further suggested that chronic ingestion of C.I. acid red 51 may promote thyroid tumor formation in rats via chronic stimulation of the thyroid by TSH.

Two-year feeding studies in dogs have been reported (Hansen *et al.*, 1973b). Groups of three male and three female beagle dogs were fed diets containing C.I. acid red 51 at levels of 0, 0.5, 1, or 2% for 2 years. No statistically significant increases in tumors were observed in the exposed animals. The number of animals and the duration of the study were inadequate for the evaluation of the carcinogenic potential of C.I. acid red 51 in the beagle dog.

Preliminary evaluation of carcinogenicity and exposure data:

C.I. acid red 51 has not been placed on the candidate list. This compound was associated with a statistically significant increase in the incidence of benign thyroid tumors in male Charles River CD rats at the highest dose tested (Borzelleca *et al.*, 1987). However, in a similar feeding study no increased tumor incidence was observed in male or female Osborne-Mendel rats (Hansen *et al.*, 1973a). C.I. acid red 51 is generally negative in gene mutation tests, but did increase micronuclei and chromosomal aberrations *in vitro*.

There is a **HIGH** level of **concern over the extent of exposure** to C.I. acid red 51, since it is used as a dye in food, drugs and cosmetics and is likely to be consumed by the general population.

References

Borzelleca JF, Capen CC, Hallagan JB (1987). Lifetime toxicity/carcinogenicity study of FD&C Red No. 3 (erythrosine) in rats. *Food Chem Toxicol* **25**(10):723-733.

Brown JP, Roehm GW, Brown RJ (1978). Mutagenicity testing of certified food colors and related azo, xanthine and triphenylmethane dyes with the *Salmonella*/microsome system. *Mutat Res* **56**:249-271.

Cameron TP, Hughes TJ, Kirby PE, Fung VA, Dunkel VC (1987). Mutagenic activity of 27 dyes and related chemicals in the *Salmonella*/microsome and mouse lymphoma TK +/- assays. *Mutat Res* **189**:223-261.

Collins TFX, Long EL (1976). Effects of chronic oral administration of erythrosine in the mongolian gerbil. *Food Cosmet Toxicol* **14**:233-248.

FDA (1998). Food color facts. Internet homepage of Food and Drug Administration at www.fda.gov.

Hansen WH, Davis KJ, Graham SL, Perry CH, Jacobson KH (1973a). Long-term toxicity studies of erythrosine. II. Effects on haematology and thyroxine and protein-bound iodine in rats. *Food Cosmet Toxicol* **11**:535-545.

Hansen WH, Zwickey RE, Brouwer JB, Fitzhugh OG (1973b). Long-term toxicity studies of erythrosine. I. Effects in rats and dogs. *Food Cosmet Toxicol* **11**:527-534.

Haveland-Smith RB, Combes RD, Bridges BA (1981). Studies on the genotoxicity of some fluorescein dyes. *Mutat Res* **88**(1):1-15.

Ishidate Jr. M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A (1984). Primary mutagenicity screening of food additives currently used in Japan. *Food Chem Toxicol* **22**:623-636.

Jennings AS, Schwartz SL, Balter NJ, Gardner D, Witorsch RJ (1990). Effects of oral erythrosine (2',4',5',7'-tetraiodofluorescein) on the pituitary-thyroid axis in rats. *Toxicol Appl Pharmacol* **103**:549-556.

Lakdawalla AA, Netrawali MS (1988). Erythrosine, a permitted food dye, is mutagenic in the Bacillus subtilis multigene sporulation assay. *Mutat Res* **206**:171-176.

Lin GHY, Brusick DJ (1986). Mutagenicity studies on FD&C Red No. 3. *Mutagenesis* **1**:253-259.

Lück H, Wallnofer P, Bach H (1963). Food additives and mutagenic effects. VII. Investigations of some xanthene dyes for mutagenic effects in E. coli. *Pathol Microbiol* (Basel) **26**:206-224.

Rogers CG, Boyes BG, Matula TI, Héroux-Metcalf C, Clayson DB (1988). A case report: A multiple end-point approach to evaluation of cytotoxicity and genotoxicity of erythrosine (FD and C Red No. 3) in a V79 hepatocyte-mediated mutation assay. *Mutat Res* **205**:415-423.

Umeda M (1956). Experimental study of xanthene dyes as carcinogenic agents. *Gann* **47**:51-78.

CARCINOGENICITY DATA SUMMARY: CHLORINATED PARAFFINS (AVERAGE CHAIN LENGTH: C_{23}, 43% CHLORINE BY WEIGHT)

Chlorinated paraffins (average chain length: C_{23}, 43% chlorine by weight) (CAS No. 108171-27-3, for chloroalkanes C_{22-26}) are used as extreme-pressure lubricant additives, as flame retardants in rubber, plastics and paints, and as secondary plasticizers, primarily in polyvinyl chloride. IARC (1990) reviewed the evidence for carcinogenicity of chlorinated paraffins (C_{23}, 43% chlorine) and determined that there was limited evidence of carcinogenicity in experimental animals and inadequate evidence of carcinogenicity in humans.

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to chlorinated paraffins (C_{23}, 43% chlorine) were found in an earlier search by IARC (1990) or more recently by OEHHA.

Animal bioassays

1. Rat 103-week gavage studies: NTP, 1986. Male and female Fischer 344/N rats (50/sex/group) were administered 0, 1875 or 3750 mg/kg body weight (males) and 0, 100, 300 or 900 mg/kg body weight (females) chlorinated paraffin (C_{23}, 43% chlorine) by oral gavage in corn oil five days per week for 103 weeks. The incidence of pheochromocytomas of the adrenal medulla was increased in females (1/50 in controls and 4/50, 6/50 and 7/50 in low-, mid- and high-dose groups, respectively) (p=0.046, significant by incidental trend test, but not by Fisher exact test). No increased incidences in tumors were reported in male rats compared to controls. NTP concluded that there was equivocal evidence for carcinogenicity in female rats and no evidence for carcinogenicity in male rats.

2. Mouse 103-week gavage studies: NTP, 1986. Male and female $B6C3F_1$ mice (50/sex/group) were administered 0, 2500 or 5000 mg/kg body weight chlorinated paraffin (C_{23}, 43% chlorine) by oral gavage in corn oil five days per week for 103 weeks. The incidence of malignant lymphomas was significantly increased in males (6/50 in controls versus 12/50 and 16/50 in low- and high-dose groups, respectively) (p = 0.009, life-table test for trend; p = 0.011, incidental tumor test for trend). The combined incidences of hepatocellular adenomas and carcinomas in high-dose females showed a marginal increase (10/50 at the high-dose versus 4/50 in controls and 3/49 at the low-dose) but the trend was not significant. NTP concluded that there was clear evidence for carcinogenicity in male mice and equivocal evidence for carcinogenicity in female mice. However, NTP noted that the low survival rate in females may have decreased the potential of the study to detect a carcinogenic effect. The NTP Board of Scientific Counselors' Technical Reports Review Subcommittee and Associated Panel of Experts were far from unanimous in the conclusion that chlorinated paraffins (C_{23}, 43% chlorine) showed clear evidence for carcinogenicity in male mice. It was stated that malignant lymphoma is one of the more variable tumors and has a viral origin in many cases. In defense of the 'clear evidence of carcinogenicity' ranking, it was stated that both low- and high-dose incidences of the tumor were above the historical control range. The decreased survival in female mice (2-year survival was 21/50, 22/50 and 16/50 for control, low- and high-dose animals) due to utero-ovarian infection may have limited the sensitivity of the study, suggesting that the hepatocellular tumors in treated females may have shown a significant trend if more of the mice had survived longer.

Other relevant data

Chlorinated paraffins (C_{23}, 43% chlorine) were not mutagenic in *S. typhimurium* strains TA100, TA1535, TA97, or TA98 in the presence or absence of a metabolic activation system (NTP, 1986). Birtley *et al.*, (1980) also found that Cereclor 55® (C_{20-30}, 42% chlorine) was not mutagenic with or without metabolic activation in *S. typhimurium* and that the material did not induce morphologic transformation of BHK cells *in vitro*. Chlorinated paraffins (C_{20-30}, 43% chlorine) did not induce chromosomal aberrations in rat bone marrow when given by gavage at toxic doses of up to 5 g/kg body weight per day for 5 days (Serrone *et al.*, 1987).

Preliminary evaluation of carcinogenicity and exposure data:

Although chlorinated paraffins (C_{23}, 43% chlorine) did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern. This is based on the clear evidence of an increase in malignant lymphoma in male mice, and equivocal evidence of carcinogenicity in female mice and rats. There is no evidence for carcinogenicity in male rats, and findings in short-term mutagenicity tests are negative.

There is a **HIGH** level of **concern over the extent of exposure,** because of the widespread use of chlorinated paraffins in industry. Production in the U.S. in 1987 was 45,000 tons. An estimated 1.5 million workers were potentially exposed in 1972-1974 in the U.S. (IARC, 1990); the NIOSH 1983 National Exposure Survey estimated that 2350 employees in the U.S. were potentially exposed across 245 facilities (RTECS, 1997).

References

Birtley RDN, Conning DM, Daniel JW, Ferguson DM, Longstaff E, Swan AAB (1980). The toxicological effects of chlorinated paraffins in mammals. *Toxicol Appl Pharmacol* **54**:514-525.

International Agency for Research on Cancer (IARC, 1990). Chlorinated Paraffins. *IARC Monographs on the Evaluation of Carcinogenic Risk to Humans: Some Flame Retardants and Textile Chemicals, and Exposures in the Textile Manufacturing Industry*. Vol. 48, p. 55-72. IARC, Lyon, France.

National Toxicology Program (NTP, 1986). Toxicology and carcinogenesis studies of chlorinated paraffins in F344/N rats and B6C3F1 mice (gavage studies). NTP-TR-305, NIH/PUB-86-2561.

Registry of Toxic Effects of Chemical Substances (RTECS, 1997). Database produced by the U.S. National Institute for Occupational Safety and Health. Version date 1/97.

Serrone DM, Birtley RDN, Weigand W, Millischer R (1987). Toxicology of chlorinated paraffins. *Food Chem Toxicol* **25**:553-562.

CARCINOGENICITY DATA SUMMARY: DICLOFOP-METHYL

Diclofop-methyl (methyl 2-(4-(2,4-dichlorophenoxy)phenoxy)propionate; Hoelon 3EC; CAS No. 51338-27-3) is a post-emergence chlorophenoxy herbicide used for the control of broad leaf plants in row crops (wheat, barley, lentil, flax and peas), as a defoliant, and for general brush control. In 1990, 53,804 lbs of diclofop-methyl were reportedly used agriculturally in California (Pease *et al*., 1993). California usage estimates in 1994 were 38,276 lbs. (CDPR, 1994). Some occupational exposure may be expected, but general population exposure should be low, primarily from food residues.

The US EPA Office of Pesticide Programs has classified diclofop-methyl in Group C as to its carcinogenicity (possible human carcinogen) and assigned a cancer potency (Q*) of 0.23 $(mg/kg\text{-}day)^{-1}$ based upon significantly increased combined liver tumors in male and female mice (US EPA, 1996). IARC concluded there was limited evidence for a carcinogenic effect from occupational exposures to chlorophenoxy herbicides, although diclofop-methyl was not among those specifically detailed in the evaluation (IARC, 1986).

Carcinogenicity Data available:

Epidemiological studies

No data regarding the carcinogenicity of diclofop-methyl to humans has been located in the literature.

Animal bioassays

The studies below were summarized based upon findings reported in the US EPA Tox Oneliner (1994) and CDPR (1996) evaluations of the data. In addition, a 15 month dog study was reported (Hoechst, 1977), but the duration and size of this experiment were inadequate for evaluation of carcinogenicity.

1. Mouse long-term feeding studies: Hoechst, 1978a. NMRKF (SPF 71) strain mice (130/sex/dose, plus 260/sex in control group) were fed diets containing 0, 2, 6.3, or 20 ppm diclofop-methyl for 102-104 weeks. Groups of 15 animals/sex were killed as an interim sacrifice at 88 weeks. Liver cell nodules (classified, in part, as benign hepatomas and, in part, as their pre- or early stages) were significantly increased in males in the high-dose group (10/243, 6/120, 4/119, and 28/120 in the control, low-, mid-, and high-dose groups, respectively; $p<<0.01$) and in females in the high-dose group (0/225, 0/112, 0/115, 9/118; $p<<0.01$). The incidence of hepatocellular carcinoma was not reported, although in the original study summary of the preliminary findings it was stated that the "number and distribution pattern of sporadically occurring benignant [sic] and malignant tumors suggested no cancerogenic properties." Liver damage observed in mice (particularly males) was attributed to peroxisome proliferation. Hemangioendotheliomas of the liver were significantly increased among female mice in the two highest dose groups (1/225, 2/112, 5/115, 5/118; $p<0.02$).

2. Rat long-term feeding studies: Hoechst, 1978b. SPF Wistar rats (90/sex/group) were fed diet containing 0, 2.0, 6.3, or 20 ppm diclofop-methyl for 2 years. No tumor formation or other adverse effects were reported. A US EPA evaluation cited inadequate doses in a tracking report from the Office of Pesticide Programs (Whiting, 1997). CDPR (1996) also indicated that the high dose was inadequate for chronic testing.

Other relevant data

Tests for the genotoxicity of diclofop-methyl are generally negative and include the *in vivo* dominant lethal test in mice, mouse micronucleus assay, recombination/conversion assay in *Schizosaccharomyces pombe*, *in vivo* cytogenetic assay in Chinese hamsters, gene conversion/mitotic recombination assays in *Saccharomyces cerevisiae*, unscheduled DNA synthesis assay in mammalian cells, and HPGRT assay in V79 cells (reviewed in US EPA, 1994). One dominant lethal assay in male rats (12/group) intubated with diclofop-methyl produced an increased mean number of dead implants at 50 mg/kg (the higher of 2 doses) (CDPR, 1996; citing Central Institut voor Veodingsonderzoek, 1975).

15-month feeding studies in dogs have been described (Hoechst, 1977). Beagle dogs (6/sex/group) were fed diet containing 0, 8, 25, or 80 ppm diclofop-methyl for 15 months. In the 25 ppm dose group, one female dog developed

a lymphosarcoma. No other tumors were reported. The duration and size of the study are inadequate for the evaluation of the carcinogenicity of diclofop-methyl to beagle dogs.

Preliminary evaluation of carcinogenicity and exposure data

Although diclofop-methyl did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern associated with the development of benign liver tumors (hepatomas in both sexes and hemangiomas in females) in the NMRKF mouse strain. There is little or no indication that diclofop-methyl is genotoxic.

There is a **HIGH** level of **exposure concern** regarding diclofop-methyl because it is a pesticide in current use providing the potential for both public and occupational exposures.

References

California Department of Pesticide Regulation (CDPR, 1996). Medical Toxicology Branch: Summary of Toxicology Data for Diclofop-methyl. Nov. 25, 1996.

Central Institut voor Veodingsonderzoek (1975). Dominant lethal assay with HOE 23408 OH in male albino rats. Report No. R 4869. 11/75.

Hoechst (1977). Report on a repeated-dose (15 months) oral toxicity study fp HOE 23408 O H AT003 in beagle dogs. Report No. 809/77, 8/17/77. Reported in Pharm Res Toxicol (1990).

Hoechst (1978a). Toxicity and tumorigenicity of HOE 23408 O H AT003 in mice during dietary administration for 2 years. Report No. 448/78, 7/19/78. Reported in *Pharm Res Tox* (1978) 539.

Hoechst (1978b). Combined chronic toxicity and tumorigenicity study with HOE 23408 O H AT003 in rats after dietary administration for two years. Report No. 449/78, 7/19/78. Reported in *Pharm Res Tox* (1978) 538, 543.

International Agency for Research on Cancer (IARC, 1986). Occupational exposures to chlorophenoxy herbicides. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Halogenated Hydrocarbons and Pesticide Exposures*. Vol. 41, p. 357-95. IARC, Lyon, France.

Pease WS, Morello-Frosch RA, Albright DS, Kyle AD, Robinson JC (1993). Preventing pesticide-related illness in California agriculture. *Strategies and priorities. An Environmental Health Policy Program Report*. Center for Occupational and Environmental Health, School of Public Health, University of California, Berkeley.

US Environmental Protection Agency (US EPA, 1994). Office of Pesticides. Tox Oneliner for Diclofop-methyl. Cleared for release Apr. 25, 1994.

US Environmental Protection Agency (US EPA, 1996). Memorandum from WL Burnam, Science Analysis Branch, Health Effects Division regarding the Office of Pesticide Programs List of Chemicals Evaluated for Carcinogenic Potential. July 15, 1996.

US Environmental Protection Agency (US EPA, 1997). Memorandum from RJ Whiting of the OPPTS/OPP/HED/SAB/PRS, Washington, DC, regarding the Office of Pesticide Programs Reference Dose Tracking Report. Feb. 25, 1997.

CARCINOGENICITY DATA SUMMARY: DILTIAZEM

Diltiazem (CAS No. 42399-41-7) is a drug which functions as a calcium (Ca^{+2}) channel blocker to control high blood pressure. The chemical structure of diltiazem is described as the following: 1,5-benzothiazepin-4(5H)-one, 2,3-dihydro-3-(acetyloxy)-5-(2-(dimethylamino)ethyl)-2-(4-methoxyphenyl)-monohydrochloride, cis-(+)-.

Carcinogenicity Data available:

Epidemiological studies

1. Prospective cohort study: Pahor *et al.*, 1996a. A study investigating potential cancer risks with the use of short-acting Ca^{+2} channel blockers (diltiazem, verapamil, or nifedipine) was conducted on a group of hypertensive patients age 71 or over with no prior history of cancer. Patients given Ca^{+2} channel blockers to control hypertension (n=202) were compared to patients given -blockers (n=424). Patients were enrolled in the cohort in 1988 and followed through 1992. A relative risk of 2.02 (95% CI 1.16-3.54) for all cancers was observed for the patients receiving Ca^{+2} channel blockers compared to those receiving -blockers. Relative risks for patients receiving diltiazem (n=76) versus those taking -blockers was 1.40 (95% CI 0.59-3.28).

2. Retrospective cohort study: Pahor *et al.*, 1996b. The study included 5052 people age 71 or older who lived in 3 regions of Massachusetts, Iowa and Connecticut and were enrolled between 1988 and 1992. Of the 451 individuals identified as taking Ca^{+2}-channel blockers for hypertension, three drugs predominated, namely diltiazem (n=184), verapamil (n=118) and nifedipine (n=146). Incidence of cancer was assessed by reviewing hospital discharge records and cause of death on death certificates. Potential confounding variables were assessed including disability, smoking, alcohol, blood pressure, body-mass index, use of other drugs, and co-morbidity. The hazard ratio for cancer (all sites) associated with use of Ca^{+2}-channel blockers (47 cases, 1549 person-years) was 1.72 (95% CI 1.27-2.34) after adjusting for confounders. A significant dose-response relationship was also reported. The hazard ratio for diltiazem alone (1.22) was not statistically significant and was somewhat lower than that for verapamil or nifedipine.

3. Retrospective cohort study: Olsen *et al.*, 1997. Enrolled in the study were 17,911 people from a county in Denmark who had received at least one prescription of Ca^{+2} channel blockers between 1991 and 1993. Cancer occurrence and rates were determined from files from the Danish Cancer Registry. The follow-up period was only 3 years, during which time 412 cancer deaths were recorded in the cohort compared with 414 expected (Standard incidence ratio 1.00, 95% CI 0.90-1.10). There was no indication of excess risk in a subgroup of likely long-term users of specific drugs, including diltiazem.

4. Nested case-control study: Jick *et al.*, 1997. Cohorts of individuals taking Ca^{+2}-channel blocking agents, angiotensin-converting-enzyme (ACE) inhibitors and -blockers were identified. All cases of cancer diagnosed in 1995 (446 cases) were matched with controls (n=1750) in a nested case-control analysis. Cancer risk of patients receiving Ca^{+2}-channel blockers and ACE inhibitors were compared to those receiving β-blockers. A relative risk of 1.27 (95% CI 0.98-1.63) was observed for persons receiving calcium-channel blockers compared to those receiving -blockers. A non-statistically significant increase in relative risk of cancer was observed for diltiazem alone (RR=1.33, relative to those receiving -blockers). There was no increase in cancer risk with increasing duration of Ca^{+2} channel blocker use, nor was there a statistically significant increased risk associated with Ca^{+2} channel blocker use and specific cancer sites.

Animal bioassays

1. Rat 24-month studies: unpublished, as described in PDR, 1998a. Rats were administered oral doses of up to 100 mg/kg-day diltiazem for 24-months. No evidence of carcinogenicity was reported.

2. Mouse 21-month studies: unpublished, as described in PDR, 1998a. Mice were administered oral doses of up to 30 mg/kg-day diltiazem for 21-months. No evidence of carcinogenicity was reported.

Other relevant data

There were no mutagenic responses *in vitro* or *in vivo* in mammalian cell assays or *in vitro* in bacteria (PDR, 1998a). Current hypotheses on possible mechanisms of tumorigenesis by Ca^{+2}-channel blocking agents such as diltiazem include alteration of apoptosis or reduction of intracellular calcium leading to increased cancer risk (Pohor *et al.*, 1996a and 1996b; Olsen *et al.*, 1997).

Two other common Ca^{+2}-channel blocking drugs, verapamil and nefedipine, were not found to be carcinogenic in animal studies or mutagenic in short-term tests. In an 18-month carcinogenicity study, rats were fed verapamil hydrochloride at a dose 6-fold higher than the recommended human dose, but not the maximum tolerated dose. No tumorigenic potential was observed (PDR 1998b). Rats were administered verapamil in the diet for 2 years at doses of 10, 35 and 120 mg/kg body weight per day (approximately 1, 3.5 and 12 times the recommended human dose, respectively). No evidence of carcinogenicity was observed (PDR 1998b). Nefidipine, administered orally to rats for 2 years, was not carcinogenic (PDR 1998c). Verapamil was negative in the Salmonella mutagenicity test (PDR 1998b).

Preliminary evaluation of carcinogenicity and exposure data:

Although diltiazem and other Ca^{+2}-channel blocking agents did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern associated with the drugs. This is because an increased risk of cancer has been associated with use of Ca^{+2}-channel blocking agents, compared to the use of other drugs for the treatment of hypertension (e.g. -blockers), in 3 separate epidemiological studies. However, the increase was statistically significant only in two studies, and only when use of all Ca^{+2}-channel blocking agents were compared, not just diltiazem alone. One epidemiological study showed no association of increased cancer risk with use of calcium channel blocking agents. Long-term animal carcinogenicity studies in rats or mice for diltiazem, or other Ca^{+2}-channel blocking drugs commonly used to treat hypertension, were negative. Negative findings for genotoxicity were also reported for diltiazem.

There is a **HIGH** level of **concern over the extent of exposure** because of the widespread use of diltiazem for the treatment of high blood pressure.

References

Jick H, Jick S, Derby LE, Vasilakis C, Wald Myers M, Meier CR (1997). Calcium-channel blockers and risk of cancer. *Lancet* **349**(9051):525-528.

Olsen JH, Sorensen HT, Friis S, McLaughlin JK, Steffensen FH, Nielsen GL, Andersen M, Fraumeni JF, Olsen J (1997). Cancer risk in users of calcium channel blockers. *Hypertension* **29**(5):1091-1094

Pahor M, Guralnik JM, Salive ME, Corti M-C, Carbonin P (1996a). Calcium-channel blockage and incidence of cancer in aged populations. *Am J Hypertension* **9**:695-699.

Pahor M, Guralnik JM, Ferrucci L, Corti MC, Salive ME, Cerhan JR, Wallace RB, Havlik RJ (1996b). Calcium-channel blockade and incidence of cancer in aged populations. *Lancet* **348**(9026):493-495.

Physician's Desk Reference (PDR, 1998a). Cardizem CD, p. 1204.

Physician's Desk Reference (PDR, 1998b). Isoptin SR, p. 1360.

Physician's Desk Reference (PDR, 1998c). ADALAT®, p. 600.

CARCINOGENICITY DATA SUMMARY: FD&C BLUE NO. 2

FD&C blue no. 2 (indigotine; CAS No. 860-22-0; 3,3'-dioxo-(delta(sup 2,2')-biindoline)-5,5'-disulfonic acid, disodium salt) is a food dye. FD&C No. 2 is one of nine synthetic color additives permitted for direct addition into food (FDA, 1993). FD&C No. 2 is used in baked goods, cereals, snack foods, ice cream, confections, and cherries (FDA, 1993). FD&C No. 2 is on the Food and Drug Administration's list of chemicals generally regarded as safe (GRAS). FDA estimates that human consumption is 0.002895 mg/kg-day per capita, assuming that 10% of the population consumes all of the colorant (FDA, 1997). FDA has set an acceptable daily intake for FD&C No. 2 at 5 mg/kg-day (FDA, 1997). The FDA Carcinogen Assessment Committee determined in October 1982 that the dye was not carcinogenic (FDA, 1997).

Carcinogenicity Data available:

Epidemiological studies

No human carcinogenicity studies were found in the literature.

Animal bioassays

1. Mouse long-term oral studies: Borzelleca and Hogan, 1985. Male and female Charles River CD -1 mice (24/dose/sex) were fed 0, 0.5, 1.5 or 5.0% of FD&C no. 2 in feed for 23 months. Treated animals did not show statistically significant incidences of tumors relative to controls.

2. Rat long-term diet studies: Oettel *et al.*, 1965. Wistar rats (35 male, 46 female) were fed FD&C blue 2 in the diet at a concentration of 1.0% for 2 years. No dose-related increases in tumor incidence relative to controls were observed.

3. Rat long-term diet studies: Hansen *et al.*, 1966. Male and female Osborne-Mendel rats (12/sex/dose) fed FD&C blue no. 2 in diet at 0, 0.5, 1.0, 2.0 or 5.0% for 2 years. There were no statistically significant increases in incidences of tumors in dosed rats relative to controls.

4. Rat long-term diet studies: Borzelleca *et al.*, 1985. Male and female CD rats were fed the dye at concentrations of 0, 0.5, 1.0, or 2.0% in the diet for 29 months in females and 30 months in males. Incidence of brain gliomas were elevated in high-dose males (7/71) versus the controls (4/140, $p<0.05$); the gliomas were malignant in the dosed rats but benign in the controls. The occurrence of gliomas was discounted by the authors because "none of the criteria for determining the neurocarcinogenic potential of chemical substances was met." Incidence of malignant mammary tumors was increased in high-dose males (3/51) relative to controls (0/114) ($p<0.05$); however, the authors did not believe that this observation was dose-related since the incidence of benign mammary tumors in the control group was 4/114 and benign tumors in the high dose group was 0/51. The incidence of mammary gland neoplasms (carcinoma and adenoma) in the female rats was not statistically significantly increased in the high-dose group 15/65 (23%) versus 22/135 (16%) in the controls.

5. Mouse long-term injection studies: Hansen *et al.*, 1966. 100 C3h and 100 C57 mice were administered subcutaneous injections containing 2.5 mg of the dye once per week for 2 years. No increased incidence of tumors were reported.

6. Rat 7-month s.c. injection study: Oettel *et al.*, 1965. Subcutaneous injections of 0.5 or 1.0 mL of a 0.5, 1.0 or 2.0% solution of dye were administered twice weekly for 7 months in Sprague-Dawley rats. No increased incidences of tumors were observed.

7. Rat long-term injection studies: Hansen *et al.*, 1966. Osborne-Mendel rats were given with weekly subcutaneous injections of 20 mg of the dye for 2 years. Fourteen of 80 Osborne-Mendel rats had injection-site tumors, whereas one of 80 control rats had an injection-site tumor.

Other relevant data

No mutagenic activity was detected in a *Salmonella*-based assay employing microsomal activation or in a mouse lymphoma assay (Cameron *et al.*, 1987). FD&C blue no. 2 was positive for sister chromatid exchanges and chromosomal aberrations in mice fed oral doses of the dye (RTECS, 1996).

Two-year oral studies have been reported in dogs (Hansen *et al.*, 1966). Two male and two female beagle dogs (6 months of age) were fed the dye at 1.0 or 2.0% in the diet for up to 2 years. One male and one female dog served as controls. The authors described no tumors in the dogs, but the report was lacking in detail, study duration was short, and the number of animals was small. The study is therefore unsuitable for the evaluation of carcinogenicity.

Preliminary evaluation of carcinogenicity and exposure data:

Although FD&C Blue No. 2 did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern. This is associated with the increased incidence of injection-site tumors in one study in the rat and possible increased incidence of brain gliomas and mammary tumors (a rare tumor) in male rats fed FD&C Blue no. 2. The level of concern is tempered by negative findings in 2 rat diet studies, 2 rat injection studies, and 2 mouse studies. Tests for mutagenicity in bacteria were negative, but positive findings were reported for clastogenicity (chromosomal aberrations and SCEs) *in vivo* in mice.

There is a **HIGH** level of **concern over the extent of exposure** because the chemical is widely used as a food additive.

References

Borzelleca JF, Hogan GK (1985). Chronic toxicity/carcinogenicity study of FD&C blue no. 2 in mice. *Food Chem Toxicol* **23**(8):719-722.

Borzelleca JF, Hogan GK, Koestner A (1985). Chronic toxicity/carcinogenicity study of FD&C blue no 2 in rats. *Food Chem Toxicol* **23**(6):551-558.

Cameron TP, Hughes TJ, Kirby PE, Fung VA, Dunkel VC (1987). Mutagenic activity of 27 dyes and related chemicals in the *Salmonella*/microsome and mouse lymphoma TK+/- assays. *Mutat Res* **189**:223-261.

Food and Drug Administration (FDA, 1993). Food color facts, FDA/IFIC brochure: January 1993 [6 pages]. FDA World Wide Web site at URL http\\www.fda.gov, May 1998.

Food and Drug Administration (FDA, 1997). FDA-GRAS database.

Hansen WH, Fitzhugh OG, Nelson AA, Davis KJ (1966). Chronic toxicity of two food colors, brilliant blue FCF and indigotine. *Toxicol Appl Pharmacol* **8**(1):29-36.

Oettel H, Frolberg H, Northdurft H, Wilhelm G (1965). Die Prüfung einiger synthetischer Farbstoffe auf ihre Eignung zur Lebensmittelfärbung. *Archiv Toxikol* **21**:9-29.

Registry of Toxic Effects of Chemical Substances (RTECS, 1997). Database produced by the U.S. National Institute for Occupational Safety and Health. Version date 1/97

CARCINOGENICITY DATA SUMMARY: MALATHION

Malathion [S-(1,2-dicarbethoxyethyl)-*O,O*-dimethyldithiophophate; *O,O*-dimethyl-S-(1,2-dicarbethoxy-ethyl)-phosphorodithioate; Carbophos®; Maldison®; Mercaptothion; CAS No. 121-75-5] is a pesticide with both agricultural and non-agricultural uses (including household use). One of its non-agricultural / household uses is in the treatment of head lice in children. Of nearly 1.9 million pounds used in 1991 in the U.S., approximately 0.8 million pounds were used for agriculture.

Californians would be expected to be exposed via agricultural work to malathion, urban spraying, application for the control of head lice, and residues in food. U.S. exposure estimates of 1982-84 average daily intakes were 142.2 ng/kg $_{bw}$-day for 6-11 month old infants, 232.8 ng/kg$_{bw}$-day for 2-year old children; 74.8, 61.8 and 53.9 ng/kg$_{bw}$-day for females aged 14-16, 25-30, and 60-65, respectively, and 107.1, 72.9 and 62.9 ng/kg$_{bw}$-day for males of these same ages (HSDB, 1994). According to the FDA's 1996 Residue Monitoring Report, malathion was the second most frequently occurring pesticide in total diet study foods (after DDT) and the eighth most frequently occurring pesticide in selected baby foods (9% occurrence with a range of 1 to 10 ppb) (FDA, 1998). The report also identified malathion as the most common pesticide contaminant found in animal feed with a reported median residue level of 0.1 ppm.

US EPA has classified malathion in group D (US EPA, 1994; US EPA, 1995), with additional studies requested. IARC (1983) has classified malathion in Group 3, based upon no data in humans and inadequate data in experimental animals. Additional evidence, including new bioassays, has become available since IARC's review.

Carcinogenicity Data available:

Epidemiological studies

1. Brown *et al.* (1993) report a case-control study of 173 men with multiple myeloma and 650 controls. There was a somewhat elevated odds ratio (OR=1.9) that was not statistically significant between farming-related malathion exposure and disease.

2. In an abstract report of a Canadian case-control study (n=2013) of non-Hodgkin's lymphoma (NHL), it was found that the risk of NHL was increased by 'substantial exposure' to malathion (OR=1.77, 95% CI=1.28-2.46) (McDuffie *et al.*, 1997 [abstract]).

3. Seventeen pesticide applicators (13 malathion-exposed and 4 controls) were evaluated for micronuclei in lymphocytes and chromosomal damage and gene mutations upon exposure of lymphocytes *in vitro* (CDHS, 1996; Windham *et al.*, 1998). The study concluded that the micronucleus frequency and mutation frequency of the glycophorin-A variant in red blood cells were not significantly different among malathion-exposed pesticide applicators compared to controls, although it was recognized that the sample size was small. A follow-up study the next year (pooled total, n=53) also showed no significant differences. *In vitro* studies showed increased micronucleated lymphocytes at cytotoxic doses of malathion (75 and 100 μg/ml) (CDHS, 1996).

4. Chromosomal aberrations were reported to be significantly increased among 14 people poisoned with malathion relative to healthy controls (van Bao *et al.*, 1974; as reported in IARC, 1983). There was no evidence of a dose-response relationship. IARC (1983) noted that the inappropriateness of the control group did not permit the establishment of a causal relationship.

Animal bioassays

1. Rat 2-year feeding studies: Daly, 1996a; as reviewed by CDPR, 1996. F344 rats (90/sex/dose) were fed diet containing 0, 100, 500, 6000, or 12,000 ppm malathion (99.4% pure) for 2 years. The 100 ppm dose group was reduced to 50 ppm on day 113 of the study. An interim kill of 35/sex/dose was conducted at 12 months. An increase in incidence of hepatocellular adenomas and carcinomas was observed in females in the two highest dose-groups. The incidence of nasoturbinal adenomas was increased among males in the two highest dose groups.

2. Rat 2-year feeding studies: Food and Drug Research Laboratories, 1980. Sprague-Dawley rats (50/sex/dose) were fed diet containing 0, 100, 1000, or 5000 ppm malathion for 2 years. No carcinogenic effects were observed.

3. Rat 103-week feeding studies: NCI, 1979b. F344 rats (49-50/sex/dose) were fed diet containing 0, 2000, or 4000 ppm malathion for 103 weeks followed by 2-3 weeks of observation. There was an increased incidence of pheochromocytoma in low-dose males but the effect was not dose-dependent. No carcinogenic effect was observed in the female rats. NCI concluded that "under the conditions of this bioassay, malathion was not carcinogenic in male or female rats, but the females may not have received a maximum tolerated dose." The conclusions of this study were not revised following a re-examination of the histopathology of the animal tissues by NTP (Huff *et al.*, 1985).

4. Rat 80-week feeding studies: NCI, 1978 as reviewed by IARC, 1983, and CDPR, 1996. Osborne-Mandel rats (50/sex/dose) were fed diet containing time-averaged doses of 4700 or 8150 ppm malathion for 80 weeks followed by a 33-week observation period. Control groups consisted of 15 rats/sex matched controls and a pooled control group of an additional 40 rats/sex. A slightly increased incidence of thyroid follicular cell adenomas and carcinomas was observed in females (as reviewed by IARC) and in males (as reviewed by CDPR). NCI stated that the "thyroid tumors were not considered to by associated with the administration of malathion". NCI concluded that "under the conditions of this bioassay, there was no clear evidence of the association of the tumor incidence with the administration of malathion to Osborne-Mendel rats". The conclusions of this study were not revised following a re-examination of the histopathology of the animal tissues by NTP (Huff *et al.*, 1985).

5. Rat 2-year feeding study: Hazleton and Holland, 1953. Male rats (20/dose) were fed diet containing 0, 100, 5000 ppm malathion for 2 years. No carcinogenic effects were reported, although IARC (1983) noted that the number of animals was small and the reporting in the study was incomplete.

6. Mouse 18-month feeding studies: Slauter, 1994. $B6C3F_1$ BR mice were fed diet containing 0, 100, 800, 8000, or 16,000 ppm malathion technical (96.4% pure) for 18 months. An interim kill of 10 animals/sex was conducted at 12 months. The terminal incidences of hepatocellular adenomas in male mice were 1/50 (control), 6/51, 2/48, 13/54, and 49/50 in increasing dose groups, and hepatocellular carcinomas were 0/50 (control), 6/51, 2/48, 6/54, and 1/50. Among female mice, the terminal incidences of hepatocellular adenomas were 0/55 (control), 1/52, 0/52, 9/52, and 42/51, and for hepatocellular carcinomas were 1/55 (control), 0/52, 2/52, 1/52, and 2/51. The incidences of hepatocellular adenomas were statistically significantly increased among male and female mice in the two highest dose groups with a positive trend in both sexes as well. The incidences of hepatocellular carcinoma were significantly increased among male mice in the 100 and 8000 ppm dose-groups, although the trend test showed no significant association with dose. Hepatocellular hypertrophy was observed in all male and female mice in the two highest dose groups.

7. Mouse 80-week feeding studies: NCI, 1978; as reviewed by IARC, 1983, and CDPR, 1996. B6C3F1 mice were fed diet containing 8000 or 16,000 ppm malathion for 80 weeks followed by a 14-15 week observation period. Control groups of 10/sex were observed for 95 weeks and pooled control of 50/sex were also included. Among male mice, combined neoplastic nodules with hepatocellular carcinomas were increased in the high dose group and the groups showed a positive trend by the Cochran-Armitage trend test (2/10, matched controls; 8/49, pooled controls, 7/48, low-dose; 17/49, high-dose; p=0.031 vs. pooled controls). When adjusted for survival to 52 weeks, neither the incidence test nor the trend test were positive when compared to the matched control group. Female mice showed no increased incidence of tumors. NCI also noted that the incidences reported in the study were within the range of some historical control groups (35-40%). The NCI concluded that "under the conditions of this bioassay, there was no clear evidence of the association of the tumor incidence with the administration of malathion to…B6C3F1 mice".

Other relevant data

An oxygen analogue, metabolite, and common contaminant of malathion, malaoxon, has been tested for carcinogenicity in a two year bioassay (NCI, 1979a). F344 rats and $B6C3F_1$ mice (50/sex/dose) were fed diet

containing 0, 500, or 1000 ppm malaoxon (>95% purity; contaminants unspecified) for 103 weeks followed by up to 2 weeks of observation. Among female rats, there was a significant increase in C-cell adenomas or carcinomas of the thyroid, although the incidence in the treated group was within the range of historical controls (the control group incidence was abnormally low). NCI concluded that "under the conditions of this bioassay malaoxon was not carcinogenic for F344 rats or $B6C3F_1$ mice." A 1985 NTP histopathological re-examination of this study led to a revision of the conclusion to a statement that "there was equivocal evidence of carcinogenicity for malaoxon in male and female F344 rats as indicated by an increased incidence for C-cell neoplasms of the thyroid gland." (Huff *et al.*, 1985).

Fischer 344 rats (85/sex/dose) were fed diet containing 0, 20, 1000, or 2000 ppm malaoxon for at least 24 months (Daly, 1996b; as reviewed by CDPR, 1996). Satellite groups of 15 animals/sex were sacrificed at 3, 6, and 12 months. No oncogenic effects were observed.

Dog 1-year feeding studies have been reported (Tegeris Laboratories, 1980). Beagle dogs (6/sex/dose) were fed diet containing 0, 62.5, 125, or 250 mg/kg-day malathion for one year. No carcinogenic effects were reported, although the duration of the study and the number of animals tested were inadequate for evaluation of carcinogenicity.

Cultured human lymphocytes exposed to 10-600 μg/ml malathion produced 'variable' mutagenic outcomes (deletions of HGPRT exon 3 detected by multiplex PCR) with little evidence of a dose-response. An *in vivo* exposed individual also showed this deletion (Pluth *et al.*, 1996a). Increased deletions in the hprt gene have been observed in human T lymphocytes exposed to malathion *in vitro* (Pluth et al, 1996b). Increased SCE and chromosomal aberrations have been observed in human peripheral leukocytes treated with malathion (Balaji and Sasikala, 1993). Malathion has tested positive for SCE, chromosomal aberrations and *in vitro* micronuclei in human lymphocytes (Titenko-Holland *et al.*, 1997).

Malathion has been tested several times in at least 5 strains of *Salmonella typhimurium* as well as *Escherichia coli* WP2 and has not been found to induce mutations either with or without metabolic activation (CDPR, 1996). Malathion showed increased mutagenic activity in the SOS chromotest for induction of β-galactosidase in *E. coli* PQ37 (Vencat *et al.*, 1995). Application of the compound with sodium taurocholate (forming micelles to simulate small intestine conditions) increased the activity in this assay to about 1/4 that observed with 4-nitroquinoline oxide (the positive reference standard).

Malaoxon induced sex-linked recessive lethal mutations in *Drosophila* when administered by feeding, but not injection (Foureman *et al.*, 1994). Translocations were not increased. X-chromosome linked recessive lethal mutations were also increased in another *Drosophila* assay (Kumar *et al.*, 1995).

Mice injected intraperitoneally with 30 mg/kg malathion showed increased chromosomal and metaphase aberrations (gaps, fragments, chromatid breaks and deletions) in the spleen 6-24 hours following treatment (Amer *et al.*, 1996). Results were reported to be comparable to other pesticides tested including Dursban, Sevin, Lannate, and DDT, but weaker than the positive control mitomycin C. Clastogenic effects including gaps, breaks, acentric fragments and dicentric chromosomes were observed in goat lymphocytes treated *in vitro* for 24 hours with malathion (Gupta *et al.*, 1996). An increase in micronucleated erythrocyte frequency was observed in mice treated with 229 mg/kg malathion relative to control (Emecen and Unlu, 1995). CDPR has cited negative studies for cytogenetic effects from exposure to malathion in the mouse dominant lethal mutation asssay (feeding study) and mouse bone marrow and spermatogonia metaphase spreads (i.p. injection and oral gavage studies) (CDPR, 1996). CDPR has cited negative studies for DNA damage including unscheduled DNA synthesis studies in human diploid fibroblasts and rat hepatocytes, assays for growth of DNA repair defective *Escherichia coli* and *Bacillus subtilis* strains, and mitotic crossing-over activity studies in *Saccharomyces cerevisiae* (CDPR, 1996).

Malathion has been shown to enhance the number of GST-positive foci in the liver of rats initiated with diethylnitrosamine (Hoshiya *et al.*, 1993).

Malathion belongs to the class of organophosphate pesticides. Other organophosphate compounds with structural similarity to malathion have been shown to produce cytogenetic effects (Sobti *et al.*, 1982).

Preliminary evaluation of carcinogenicity and exposure data

Although malathion did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern. This is primarily associated with the long-term studies in male and female rats and mice which show the development of liver tumors (primarily adenomas). In mice, the data show that the incidence of liver adenomas occurs to a high degree, particularly in males with almost all high-dose animals developing tumors. This level of concern is tempered by several similarly-conducted long-term studies showing no carcinogenic effect. The designated level of concern is supported by *in vitro* studies which show that malathion has the potential to cause cytogenetic damage to human and other animal cells. Bacterial genotoxicity tests have generally been negative, although there have been a few positive findings.

There is a **HIGH** level of **concern over the extent of exposure** to malathion. It is a pesticide in current use in the state of California with known human exposures.

References

Amer SM, Fahmy MA, Donya SM (1996). Cytogenetic effect of some insecticides in mouse spleen. *J Appl Toxicol* **16**:1-3.

Balaji M, Sasikala K (1993). Cytogenetic effect of malathion in *in vitro* culture of human peripheral blood. *Mutat Res* **301**(1):13-17.

Brown LM, Burmeister LF, Everett GD, Blair A (1993). Pesticide exposures and multiple myeloma in Iowa men. *Cancer Causes Control* **4**(2): 153-6.

California Department of Health Services (CDHS, 1996). The genetic toxicity of malathion: Results of an epidemiological study in agricultural workers and *in vitro* laboratory studies. Berkeley, California.

California Department of Pesticide Regulation (CDPR, 1996). Summary of toxicological data - malathion. July 30, 1986. Updated 11/18/96.

Daly IW (1996a). A 24-month oral toxicity/oncogenicity study of malathion in the rat via dietary administration. Huntingdon Life Sciences, East Millstone, NJ. Study No. 90-3641. Feb. 27, 1996.

Daly IW (1996b). 24 month oral toxicity/oncogenicity study of malaoxon in the rat via dietary administration. Huntingdon Life Sciences, East Millstone, NJ. Study No. 93-2234. April 2, 1996.

Emecen G, Unlu H (1995). The investigation of the cytogenetic effects of pesticides on mammals by micronucleus test [in Turkish]. *Turk J Biol* **19**(1): 1-9.

Food and Drug Administration (FDA, 1998). Center for Food Safety and Applied Nutrition. Food and Drug Administration Pesticide Program: Residue Monitoring 1996. January, 1998.

Food and Drug Research Laboratories (1980). The evaluation of the chronic toxicity effects of cythion administered in the diet to Sprague-Dawley rats for 24 consecutive months. *Laboratory* No. 5436. May 13, 1980.

Foureman P, Mason JM, Valencia R, Zimmering S (1994). Chemical mutagenesis testing in *Drosophila*. X. Results of 70 coded chemicals tested for the National Toxicology Program. *Environ Mol Mutagen* **23**(3): 208-27.

Gupta SC, Sahai R, Gupta N (1996). Cytogenetic effects of organophosphate pesticides on goat lymphocytes in culture. *AJAS* 9(4): 449-54.

Hazleton LW, Holland EG (1953). Toxicity of malathion. Sumary of mammalian investigations. *Arch Ind Hyg Occup Med* **8**:399-405.

Hoshiya T, Hasagawa R, Hakoi K, Cui L, Ogiso T, Cabral R, Ito N (1993). Enhancement of non-mutagenic pesticides of GST-P positive hepatic foci development initiated with diethylnitrosamine in the rat. *Cancer Lett* **72**:59-64.

Hazardous Substances Data Bank (HSDB, 1996). Micromedex, Inc. Denver, CO.

Huff JE, Bates R, Eustis SL, Haseman JK, McConnell EE (1985). Malathion and malaoxon: histopathology reexamination of the National Cancer Institute's carcinogenesis studies. *Environ Res* **37**(1): 154-73.

International Agency for Research on Cancer (IARC, 1983). Malathion. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Miscellaneous Pesticides* Vol 30, pp103-129. IARC, Lyon, France

Ito N, Hasegawa R, Imaida K, Takahashi S, Shirai T (1994). Medium-term rat liver bioassay for rapid detection of carcinogens and modifiers of hepatocarcinogenesis. *Drug Metab Rev* **26**(1-2)**:**431-442.

Kumar D, Khan PK, Sinha SP (1995). Cytogenetic toxicity and no-effect limit dose of pesticides. *Food Chem Toxicol* **33**(4): 309-14.

McDuffie HH, Cross Canada Study of Pesticides and Health Group (1997). Non-Hodgkin's lymphoma (NHL) and the pesticide hypothesis: Individual compounds [Abstract]. *Proc Ann Meet Am Assoc Cancer Res* **38**:A4210.

National Cancer Institute (NCI, 1978). Bioassay of malathion for possible carcinogenicity. TR-24. US DHEW, Washington, DC.

National Cancer Institute (NCI, 1979a). Bioassay of malaoxon for possible carcinogenicity. TR-135, US DHEW, Washington, DC.

National Cancer Institute (NCI, 1979b). Bioassay of malathion for possible carcinogenicity. TR-192, US DHEW, Washington, DC.

Pluth JM, Nicklas JA, O'Neill JP, Albertini RJ (1996a). Increased frequency of specific genomic deletions resulting from in vitro malathion exposure. *Cancer Res* **56**(10): 2393-9.

Pluth JM, Nicklas JA, O'Neill JP, Albertini RJ (1996b). Mutational spectra comparison of malathion in vitro induced hprt mutants analyzed by both multiplex PCR and cDNA sequencing. 27th Annual Scientific Meeting of the Environmental Mutagen Society, Victoria, British Columbia. *Environ Mol Mutagen* **27**:54.

Slauter RW (1994). 18-Month oral (dietary) oncogenicity study in mice. Malathion. International Research and Development Corporation. Mattawan, MI. Project ID 668-001. October 12, 1994.

Sobti RC, Krishan A, Pfaffenberger CD (1982). Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. *Mutat Res* **102**(1):89-102.

Tegeris Laboratories (1980). One-year oral toxicity study in purebred beagle dogs with AC 6,601. Report No. 85010. Feb. 10, 1987.

Titenko-Holland N, Windham G, Kolachana P, Reinisch F, Parvatham S, Osorio AM, Smith MT (1997). Genotoxicity of malathion in human lymphocytes assessed using the micronucleus assay in vitro and in vivo: A study of malathion-exposed workers. *Mutat Res* **388**(1):85-95.

US Environmental Protection Agency (US EPA, 1994). Drinking water regulations and health advisories. Office of Water, Washington, DC.

US Environmental Protection Agency (US EPA, 1995). Memorandum from SR Irene, Acting Director, Office of Pesticide Programs (OPP), re OPP's List of Chemicals Evaluated for Carcinogenic Potential. Aug. 7, 1995.

van Bao I, Szabó I, Ruzicska P, Czeizel A (1974). Chromosome aberrations in patients suffering acute organic phosphate insecticide intoxication. *Humangenetik* **24**:33-57.

Vencat JA, Shami S, Davis K, Nayak M, Plimmer JR, Pfeil R, Nair PP (1995). Relative genotoxic activities of pesticides evaluated by a modified SOS microplate assay. *Environ Mol Mutagen* **25**(1):67-76.

Windham GC, Titenko-Holland N, Osorio AM, Gettner S, Reinisch F, Haas R, Smith M (1998). Genetic monitoring of malathion-exposed agricultural workers. *Am J Ind Med* **33**(2): 164-74.

CARCINOGENICITY DATA SUMMARY: OMEPRAZOLE

Omeprazole (CAS no. 73590-58-6; 5-methoxy-2-[[(4methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole, "Prilosec") is a drug used to inhibit gastric acid secretion. It achieves this inhibition by binding to and inhibiting the proton pump protein in the secretory epithelium of the stomach. This mechanism is in contrast to other well-known inhibitors of gastric acid secretion such as cimetidine and ranitidine, which are inhibitors of the H_2 histamine receptor. It is used to treat and prevent recurrence of gastric, duodenal and esophageal ulceration, particularly in patients whose disease has proved refractory to the H_2 inhibitors, which have been longer established in clinical practice and are more widely used. Single daily oral doses in the range 10mg - 40 mg (in the form of delayed-release capsules) are used, and typically produce a reduction in gastric acid output of at least 80% over a 24 hour period. The manufacturer asserts that approximately 130 million "patient treatments" (this phrase is assumed to mean individual doses) have been administered worldwide up to January 1996 (Roberts, 1996).

Carcinogenicity Data available:

Epidemiological studies

Various clinical trials involving acute or medium-term exposures to omeprazole have been performed. Side-effects found associated with omeprazole use are primarily confined to non-specific symptoms such as headache and nausea, and are of relatively low frequency, although a small number of more serious idiosyncratic reactions have been reported (Piper, 1995). Apart from the histological evaluation of gastric endocrine cells and measurement of serum gastrin levels (an important issue for potential carcinogenic effects, as discussed below in the section on other relevant data), the studies report primarily short-term side effects related to tolerability of the treatment. In this respect, omeprazole resembles other inhibitors of gastric secretion.

The following reviews of clinical trial and patient treatment data using epidemiological methods were noted by Piper [1995]). The maximum duration of exposure appears not to have exceeded six years in any of these investigations, so these studies are of limited value in the evaluation of carcinogenic effects.

1. Solvell (1990). Incidences of serious adverse effects in several comparative clinical studies of patients taking omeprazole were reported. The total number of patients involved exceeded 19,000. Comparisons were made with other drugs prescribed for gastric ulcers (ranitidine, cimetidine). Histological studies of the gastric mucosa of patients treated with omeprazole (usual dose 20 mg/day, for up to 37 months) did not show any dose-related changes that might be related to the carcinoids observed in animal studies. In this study 248 patients were examined, who had their last biopsy taken after at least 11 months of omeprazole treatment.

2. Joelsen *et al.* (1992). Comparative clinical trials of patients taking omeprazole or H_2 receptor antagonists were reported. Short-term studies (2-8 weeks) involved 1,919 patients taking omeprazole matched to 1,572 taking ranitidine, and 899 taking omeprazole compared to 891 taking cimetidine. Long-term studies involved 859 patients treated with omeprazole for up to six years. Total exposure time amounted to 957 patient years. Adverse outcomes during long-term exposure were similar to those seen during the short-term exposure, and there were no serious adverse outcomes attributed to omeprazole therapy.

3. Lloyd-Davies *et al.* (1988). The clinical outcomes and incidences of side effects were reported in 80 patients taking omeprazole as a treatment for Zollinger-Ellison syndrome (in which extreme hypersecretion of gastric acid occurs). Adverse events (other than those related to underlying disease) were infrequent, and only two were thought to be dose-related. Several patients were found to have duodenal polyps, but these were generally present at the start of treatment. Only one polyp was found on investigation to include ECL cells: this was a mixed endocrine type which appeared to be a recurrence of a lesions removed prior to the start of omeprazole treatment. The authors stated that "There was no evidence of carcinoids in any of the gastric biopsies examined …").

4. Arnold and Koop (1989). Several clinical studies of patients taking omeprazole were summarized, in which adverse outcomes were generally similar to those seen in patients taking H_2 receptor antagonists. No serious adverse outcomes were attributed to the omeprazole therapy. Studies of serum gastrin levels during omeprazole

treatment, and one study of effects on gastric endocrine cells (Lamberts *et al*., 1988) were described. Serum gastrin increases in patients treated with omeprazole were only moderate, and did not result in gastric endocrine cell hyperplasia.

Other brief reports, in which systematic statistical analysis was not employed, were also identified:

5. Graham (1992). In this report several cases of gastric polyposis are described, which had apparently arisen during, and might be related to, omeprazole treatment. This report, and the suggestion that endoscopy be used to look for additional such findings in omeprazole-treated patients, was met with a rebuttal (Dent, 1992) which is also lacking in quantitative analysis.

6. Brunner *et al.*, 1990 [cited by Graham (1992)]. This report apparently found some histological evidence of gastric endocrine cell hyperplasia.

Animal bioassays

1. Rat long-term oral (gavage) studies (Havu, 1986). Groups of 60 male or female Sprague-Dawley rats received daily oral doses of 40, 125 or 400 µmol/kg (14.1, 44.0 or 140.8 mg/kg) omeprazole by gavage for 104 weeks. Control groups of 120 animals were dosed with the vehicle only. The surviving animals were killed at 104 weeks. Survival was reduced in the high dose male group, with 82% mortality before week 104, but not in the other males (45-48% mortality before week 104) or the females (33-49% mortality before week 104). Special efforts were taken to permit histological examination of the gastric mucosa, including the use of special stains and the preparation of some visible nodules for electron microscopy. Marked enterochromaffin-like (ECL) cell hyperplasia was observed in treated rats. Gastric ECL cell carcinoids were observed in some members of all treated groups of females. Carcinoids were also observed, at lower incidence rates, in all treated groups of males except the low-dose group. No gastric carcinoids were observed in any control animal. The increases were as follows: in males (control, low-, mid-, high-dose) 0/119, 0/60, 1/60, 6/60; in females 0/120, 13/60, 19/60, 24/60. Submucosal invasion was observed in 12 animals, but no metastasis was observed: the tumors were described by the authors as showing local malignancy. The tumors tended to be late-appearing (only 4 being identified in animals dying before week 100), and were considered to be incidental rather than fatal when examined at autopsy. The authors did not report efforts to look for tumors in tissues other than the gastric mucosa, nor were any such observations described.

2. Mouse long-term oral (gavage) studies (Havu, 1986). A similar experiment was performed with CD-1 mice. Groups of 60 males or females received daily oral doses of 40, 125 or 400 µmol/kg (14.1, 44.0 or 140.8 mg/kg) omeprazole by gavage for 78 weeks. Control groups of 120 animals were dosed with the vehicle only. The surviving animals were killed at 78 weeks. No gastric carcinoids or related ECL cell changes were observed in any exposed or control animal.

In addition to the two series of experiments noted above which are fully reported in the open literature, there are four studies which are noted by FDA (1989) on the basis of regulatory submissions to that Agency. It appears that two of these are parallel reports of the studies in Sprague-Dawley rats and CD-1 mice reported by Havu (1986). The other two appear to be additional or supplementary studies, which are described below.

3. Long-term oral (gavage) study in female Sprague-Dawley rats (FDA, 1989). The low dose of the earlier study was the high dose of this study. Dose-related increases in gastric ECL cell carcinoids were observed: 2%, 8% and 24% for low, mid and high dose groups compared to none in controls.

4. One year treatment/one year recovery study in female Sprague-Dawley rats (FDA, 1989). An unusual, uncommon primary malignant gastric tumor was observed in one rat. There is no record of this or similar tumors in historical controls.

Other relevant data

Omeprazole is inactive in bacterial mutagenicity assays and other genotoxicity tests *in vitro* or *in vivo* (Powers *et al.*, 1995; Roberts, 1996). Two reports (Burlinson *et al.*, 1991; Furihata *et al.*, 1991) indicated that omeprazole causes

unscheduled DNA synthesis in rat stomach. Other authors failed to confirm these reports, and identified possible methodological problems which may have contributed to the original observations. Powers *et al.* (1995) asserted that the finding by Burlinson *et al.* "was widely refuted".

The mode of action of omeprazole involves activation at the very low pH present in the secretory cell, to a reactive sulfinyl intermediate (McTavish *et al.*,1991). Although this intermediate reacts with and permanently inhibits the proton pump protein, there is no evidence that it is formed near DNA, or survives long enough to diffuse there.

One of the consequences of the gastric achlorhydria induced by omeprazole and other inhibitors of gastric secretion is an increase in circulating levels of the hormone gastrin. This hormone is produced by the antral region of the stomach and in the normal situation provides feedback control of acid secretion. Chronic hypergastrinemia is also a feature of certain disease states, including "pernicious anemia" (symptoms of Vitamin B_{12} deficiency), which is associated with atrophic gastritis, and the presence of certain endocrine tumors. It has been shown that chronic severe hypergastrinemia in humans results in ECL cell hyperplasia and stomach tumors of the otherwise rare type referred to as ECL cell carcinoids. This tumor type is unknown in non-human species other than the rat and one other rodent (*Mastomys natalensis*). Hypergastrinemia was considered by Havu (1986) to be the causative factor in the observation of ECL cell carcinoids in the rat after exposure to omeprazole and certain other pharmacological agents, such as loxtidine (an H_2 inhibitor). Similar observations following exposure to omeprazole, SK&F 9347 or (to a lesser extent) oxmetidine) were reported by Betton *et al.* (1988). The induction of ECL cell hyperplasia and gastric carcinoids in rodents by omeprazole and H_2 receptor antagonists was reviewed by Poynter and Selway (1991).

Other proposed causes of gastric carcinogenesis as a result of achlorhydria have involved the appearance of bacteria in the stomach (which is normally sterile due to the low pH) and activation of nitrates or nitrites by these bacteria to carcinogenic nitrosamines. In omeprazole-induced achlorhydria, endogenous formation of N-nitrosomorpholine from morpholine (but not nitrosation of thiazolidine-4-carboxylic acid) given to rats was increased (Calmels *et al.*, 1991). Also noted is the irritant and possibly carcinogenic or promoting effect of infection by *Helicobacter pylori*, a causative agent of gastric and duodenal ulcers. Further discussion of these hypotheses is presented by Piper (1995) and by McTavish *et al.* (1991). Piper (1995) also argued that risks of gastric carcinogenesis from treatment with omeprazole or H_2 antagonists were low due to the moderate degree of achlorhydria and hypergastrinemia induced by therapeutic doses, compared to the profound changes seen in the disease states known to be associated with ECL cell carcinoid appearance in humans.

Preliminary evaluation of carcinogenicity and exposure data:

Although omeprazole did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern, since tumors of an otherwise rare type have been observed in both sexes of the rat (but not in the mouse). The mechanism of induction of these tumors may involve stimulation of gastric endocrine cells via gastrin in response to the profound achlorhydria induced by omeprazole treatment. The concern is increased by the observation of similar tumors in humans, probably caused by a similar mechanism, in certain disease states. The concern is however reduced by the evidence from short or medium-term clinical trials and therapeutic use. Negative data from these studies, and measurements of the histological impact of omeprazole treatment on gastric endocrine cells in humans, have been interpreted to suggest that at least at therapeutic doses the effect of omeprazole on gastric acid secretion and gastrin secretion is not sufficiently severe to cause the appearance of carcinoid tumors. It is at present unclear whether this difference constitutes a qualitative difference in scale of effect, such that tumors would never be expected as a result of these drug treatments, or whether omeprazole should be considered a potential human carcinogen but that this risk is low provided treatment is for shorter periods and at normal therapeutic doses only.

There is a **HIGH** level of **concern over the extent of exposure.** Omeprazole is widely prescribed as one of several possible treatments for gastric, duodenal or esophageal diseases, some forms of which are fairly common. Omeprazole is apparently used in cases of more severe or recurrent disease. Prescribing guidelines previously cautioned against long-term use, although this restriction has recently been relaxed. Since it is available only as a

prescription medicine, exposure of the non-patient population and general environmental exposures are unlikely, although some occupational exposures may be anticipated for manufacturing and health care workers.

References

Arnold R, Koop N (1989). Omeprazole: long term safety. *Digestion* **44**(Suppl 1):77-86.

Betton GR, Dormer CS, Wells T, Pert P, Price CA, Buckley P (1988). Gastric ECL-Cell Hyperplasia and Carcinoids in rodents following chronic administration of H_2 antagonists SK&F 93479 and Oxmetidine and Omeprazole. *Toxicol Pathol* **16**(2):288-298.

Brunner G, Lamberts R, Creutzfeldt W (1990). Efficacy and safety of omeprazole in the long-term treatment of peptic ulcer and reflux oesophagitis resistant to ranitidine. *Digestion* **47**(Suppl 1):64-68.

Burlinson B, Morriss S, Gatehouse DG, Tweats DJ, Jackson MR (1991). Uptake of tritiated thymidine by cells of the rat gastric mucosa after exposure to loxtidine or omeprazole. *Mutagenesis* **6**:11-18.

Calmels S, Béréziat JC, Ohshima H, Bartsch H (1991). Bacterial formation of N-nitroso compounds in the rat stomach after omeprazole-induced achlorhydria. In: *Relevance to human Cancer of N-Nitroso Compounds, Tobacco Smoke and Mycotoxins*. Eds: O'Neill IK, Chen J, Bartsch H. IARC Scientific Publication No. 105, IARC, Lyon, 1991.

Dent J (1992). Gastric polyposis: onset during long-term therapy with omeprazole. *Med J Australia* **157**: 645-646.

Food and Drug Administration (FDA, 1989). Omeprazole Capsules Original NDA and NDA Amendments. Submitted by Merck & Co. 6/30/88; 10/12/88; 1/24/89.

Furihata C, Hirose K, Matsushima T (1991). Genotoxicity and cell proliferative activity of omeprazole in rat stomach mucosa. *Mut Res* **262**:73-76.

Graham JR (1992). Gastric polyposis: onset during long-term therapy with omeprazole. Med J Australia 157: 287-288.

Havu N (1986). Enterochromaffin-like cell carcinoids of gastric mucosa in rats after life-long inhibition of gastric secretion. *Digestion* **35**(Suppl 1):42-55.

Joelsen S, Joelsen B, Lindberg P *et al.* (1992). Safety experience from long term treatment with omeprazole. *Digestion* **51**(Suppl 1):93-101.

Lloyd-Davies KA, Rutguersson K, Solvell L (1988). Omeprazole in the treatment of Zollinger-Ellison syndrome: a 4 year interventa l study. *Aliment Pharmacol Ther* **2**:13-32.

Lamberts R., Creutzfeldt W, Stöckmann F (1988). Long-term omeprazole treatment in man: effects on gastric endocrine cell populations. *Digestion* **39**:126-135.

McTavish D, Buckley MM, Heel RC (1991). Omperazole. An updated review of its pharmacology and therapeutic use in acid-related disorders. *Drugs* **42**(1):138-170.

Piper DW (1995). A comparative overview of the adverse effects of antiulcer drugs. *Drug Safety* **12**(2):120-138.

Powers RE, Lawton GP, Modlin IM (1995). Genotoxicity, carcinogenicity and acid-suppressing medications. *Pharmac Ther* **65**(3):303-17.

Poynter D, Selway SAM (1991). Neuroendocrine cell hyperplasia and neuroendocrine carcinoma of the rodent fundic stomach. *Mutat Res* **248**:303-319.

Roberts GM (1996). Letter to James Stratton, OEHHA from Gary M. Roberts of Gibson, Dunn and Crutcher, lawyers. January 31st 1996.

Solvell L (1990). The clinical safety of omeprazole. *Digestion* **47**(Suppl 1):59-63.

CARCINOGENICITY DATA SUMMARY: TOCOPHEROL MIX (E-MIX 80®)

A mixture of tocopherols, known as E-mix 80® (CAS No. 1406-66-2), consists of natural tocopherols, including -tocopherol (vitamin E) and other isomers. This particular mixture (*i.e.*, E-mix 80®) has only been referred to in publications from researchers in Japan. It is assumed that this tocopherol mix is available in Japan for basic research purposes, and perhaps human consumption as a food additive and dietary supplement. Although OEHHA was unable to verify if this specific tocopherol mix is used or consumed in the U.S., mixtures of natural tocopherols are commonly found in foods and dietary supplements.

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to the specific tocopherol mix known as E-mix 80® were found in the literature. Numerous epidemiological studies conducted in diverse populations from around the world have suggested that increased consumption or increased serum levels of tocopherols protect against several forms of cancer, including lung, gastrointestinal (Knekt, 1988; Knekt *et al*., 1991; Nitta *et al*., 1994; Ingles *et al*., 1998), and prostate cancer (Patterson *et al.,* 1997; Heinonen *et al*., 1998).

Animal bioassays

-Tocopherol alone and in combination with other tocopherols has been shown in numerous studies conducted in several different experimental animal models to inhibit the formation of chemically-induced tumors. There is, however, one report of the induction of tumors in mice by a mixture of tocopherols known as E-mix 80®, and another of the induction of transplantable injection site tumors in mice and rats by natural and synthetic -tocopherol. These are described below.

1. Mouse 104 week feeding study: Nitta *et al.* 1991a. Groups of male 6HF$_1$ mice were fed E-mix 80® at either 0 or 5% in the diet for 104 weeks. The authors reported that the frequency of the "spontaneous development" of liver tumors was significantly elevated in the E-mix 80® treated animals. In the same series of experiments, groups of mice were administered a carcinogenic dose of N-nitrosodiethylamine, and then fed a diet containing either 0 or 5% E-mix 80®. In these animals, E-mix 80® was found to protect against the formation of N-nitrosodiethylamine-induced liver, lung, and upper alimentary tract tumors.

2. Mouse subcutaneous injection studies: Nitta *et al.* 1991b. Groups of 5 animals/sex/strain/treatment from two different strains of mice, NFS/N and C57BL/6N x C3H/He F$_1$, received repeated subcutaneous injections of either natural -tocopherol or synthetic dl- -tocopherol acetate, either alone, or in soya or palm oil. In females, natural -tocopherol induced transplantable tumors at the site of injection in both strains, when injected in soya oil. dl- -Tocopherol acetate injected in either soya or palm oil induced transplantable tumors at the site of injection in NFS/N mice.

3. Rat subcutaneous injection study: Nitta *et al*. 1991b. Fischer 344 rats received repeated subcutaneous injections of either natural -tocopherol or synthetic dl- -tocopherol acetate. dl- -Tocopherol acetate induced transplantable tumors at the site of injection, when injected in either soya or palm oil.

Other Relevant Data

-Tocopherol administered in the diet to male B6C3F$_1$ mice previously treated with diethylnitrosamine was shown to promote the growth of hepatic focal lesions (Kolaja *et al*., 1998). In the same study, however, vitamin E was shown to block the growth-promoting effect of dieldrin on hepatic focal lesions in mice previously treated with diethylnitrosamine.

Tocopherols protect against oxidative damage and inhibit lipid peroxidation of cellular membranes, preventing the creation of a pro-oxidant, tumor-promoting state.

Preliminary evaluation of carcinogenicity and exposure data

The tocopherol mix E-mix 80®, and other natural and synthetic tocopherols, have not been placed on the candidate list. E-mix 80® was associated with an increased "spontaneous" incidence of liver tumors, in a single experiment in male mice. In that same series of experiments, E-mix 80® inhibited the formation of N-nitrosodiethylamine-induced tumors of the liver, lung and upper alimentary tract. -Tocopherol (both natural and synthetic forms) induced transplantable injection site tumors in rats and mice. The level of concern is considerably tempered, however, by the large body of evidence in humans and experimental animals indicating that tocopherols are protective against tumor induction.

There is a **HIGH** level of **concern over the extent of exposure** to tocopherol mixtures. Although there are no data on exposure in the U.S. to E-mix 80®, natural sources of tocopherols are plentiful in the diet, and dietary supplements containing natural and synthetic tocopherols are widely used. Topical exposures also occur in the general population through the dermal application of creams, lotions, and other preparations containing tocopherols.

References

Heinonen OP, Albanes D, Virtamo J, Taylor PR, Huttunen JK, Hartman AM, Haapakoski J, Malila N, Rautalahti M, Ripatti S, Maenpaa H, Teerenhovi L, Koss L, Virolainen M, Edwards BK (1998). Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. *J Natl Cancer Inst* **18**:440-446.

Ingles SA, Bird CL, Shikany JM, Frankl HD, Lee ER, Haile RW (1998). Plasma tocopherol and prevalence of colorectal adenomas in a multiethnic population. *Cancer Res* **58**:661-666.

Knekt P (1988). Serum vitamin E level and risk of female cancers. Int J Epidemiol **17**:281-286.

Knekt P, Aromaa A, Maatela J, Aaran RK, Nikkari T, Hakama M, Hakulinen T, Peto R, Teppo L (1991). Vitamin E and cancer prevention. *Am J Clin Nutr* **53**:283S-286S.

Kolaja KL, Xu Y, Walborg EF Jr, Stevenson DE, Klaunig JE (1998). Vitamin E modulation of dieldrin-induced hepatic focal lesion growth in mice. *J Toxicol Environ Health* **53**:479-492.

Nitta Y, Kamiya K, Tanimoto M, Kagimoto O, Niwa O, Yokoro K (1991a). Effects of administration of natural vitamin E on spontaneous hepatocarcinogenesis and N-nitrosodiethylamine initiated tumors in mice. *J Toxicol Pathol* **4**:55-61.

Nitta Y, Kamiya K, Tanimoto M, Sadamoto S, Niwa O, Yokoro K (1991b). Induction of transplantable tumors by repeated subcutaneous injections of natural and synthetic vitamin E in mice and rats. *Jpn J Cancer Res* **82**:511-517.

Nitta Y, Kamiya K, Kenjiro Y (1994). Vitamin E and carcinogenesis. *J Toxicol Pathol* **7**:179-90.

Patterson RE, White E, Kristal AR, Neuhouser ML, Potter JD (1997). Vitamin supplements and cancer risk: the epidemiologic evidence. *Cancer Causes Control* **8**:786-802.

CARCINOGENICITY DATA SUMMARY: TRIADIMENOL

Triadimenol (Baytan®; CAS No. 55219-65-3) is a pesticide that is registered in California for use as a fungicide on corn, barley, oats, wheat and cotton. The California Department of Pesticide Regulation's 1994 Pesticide Use Report does not contain any information on the quantity of triadimenol used in California.

Carcinogenicity Data available:

Epidemiological studies

No epidemiological studies of cancer rates in humans exposed to triadimenol were found in the literature.

Animal bioassays

1. Rat 2-year feeding studies: US EPA, 1989. Male and female rats were given triadimenol mixed with feed for 2 years at doses of 0, 6.25, 25 and 100 mg/kg-d. No significant increases in tumor incidence were detected.

2. Mouse 2-year feeding studies: US EPA, 1989. Male and female mice were given triadimenol mixed with feed to produce doses of 0, 18, 72, or 285 mg/kg-d for 2 years. There was a significantly increased incidence of hepatocellular adenomas in females at the high dose, but not in females given lower doses or in any of the groups of males fed triadimenol.

Other relevant data

Triadimenol was not mutagenic or clastogenic in multiple short-term tests (US EPA, 1989).

Preliminary evaluation of carcinogenicity and exposure data:

Triadimenol has not been placed on the candidate list. An increase in the incidence of benign liver tumors, in the high-dose group only, was observed in female mice fed triadimenol for two years. There was no evidence of carcinogenicity in male or female rats, or in male mice fed triadimenol for two years. Triadimenol was not mutagenic or clastogenic in tests for genotoxicity.

There is a **HIGH** level of **concern over the extent of exposure** to triadimenol because it is registered for use on certain cereal crops.

References

U.S. Environmental Protection Agency (US EPA, 1989). Pesticide Fact Handbook, Triadimenol Fact Sheet 204: 628-635.

CARCINOGENICITY DATA SUMMARY: TRIMETHYLTHIOUREA

Trimethylthiourea (1,1,3-trimethyl-2-thiourea; CAS No. 2489-77-2) is used in a wide variety of industrial applications, including as a rubber vulcanization accelerator, a component of adhesives, and an intermediate in the synthesis of dyes and pharmaceuticals. Production of the chemical in the US was estimated to be more than 454 kg in 1977 (HSDB, 1997).

Carcinogenicity Data available:

Epidemiological studies

No data on the carcinogenic effects of this chemical in humans were identified.

Animal bioassays

1. Rat 77-week dietary studies: NCI, 1979. Groups of 50 male and 50 female Fischer 344 rats were fed a mixture containing 80% trimethylthiourea and 15% dimethylthiourea in the diet at either 250 or 500 ppm for 77 weeks, followed by an observation period of 29 weeks on control diet. Twenty animals of each sex were used as controls. There was a dose-related increase of follicular-cell carcinomas of the thyroid in female rats (0/17, 1/38, and 14/47 in the control, low-, and high-dose groups, respectively). The incidences of follicular-cell carcinomas and follicular-cell carcinomas and adenomas (combined) were significantly higher in the high-dose females as compared to the controls. There were no significant positive associations between compound administration and tumor incidences in male rats. NCI concluded that under the conditions of the bioassay, dietary administration of trimethylthiourea was carcinogenic to female Fischer 344 rats.

2. Mouse 77-week dietary studies: NCI, 1979. Groups of 50 male and 50 female B6C3F_1 mice were fed a mixture containing 80% trimethylthiourea and 15% dimethylthiourea in the diet at either 500 or 1000 ppm for 77 weeks, followed by an observation period of 14 weeks on control diet. Twenty animals of each sex were used as controls. There was no significant positive association between the dosage of administration and mortality in mice of either sex. For high-dose mice of both sexes, compound-related mean body weight depression was observed, indicating that the dosages of trimethylthiourea administered to these animals may have reached the maximum tolerated doses. NCI reported there was no positive association between compound administration and tumor incidences in mice of either sex. NCI concluded that under the conditions of the bioassay, there was not sufficient evidence for the carcinogenicity of the compound in B6C3F_1 mice of either sex.

3. Mouse interperitoneal injection studies: Maronpot *et al*., 1986. Groups of 10 male and 10 female Strain A/St mice were administered with trimethylthiourea by interperitoneal injection, 3 times a week for 8 weeks at doses of 0, 50, 100, or 150 mg/kg per injection. After the injections were completed, the mice were held until the end of a 16-week observation period. All of the male and female mice injected with 50 and 150 mg/kg trimethylthiourea survived until the end of the experiment and all of the animals injected with 100 mg/kg trimethylthiourea died prematurely. No lung tumors were observed in treated mice of either sex.

Other Relevant Data:

Structurally, trimethylthiourea is similar to ethylene thiourea, a tumorigen in mice. Trimethylthiourea is generally not mutagenic in genotoxic tests (results cited in Maronpot *et al*., 1986) but the compound has been shown to be positive without metabolic activation in the mouse lymphoma cell forward mutation assay (McGregor *et al*., 1988).

Preliminary evaluation of carcinogenicity and exposure data:

Although trimethylthiourea did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern associated with the induction of follicular-cell carcinomas of the thyroid in female rats. Trimethylthiourea is generally not considered to be genotoxic, but was shown to be positive in a mouse lymphoma mutation assay. Based on the structural relationship with other thioureas, trimethylthiourea is expected to have antithyroidal effects in both animals and humans.

There is **HIGH** level of **concern** over the extent of exposure since trimethylthiourea has many industrial applications and there is the potential for widespread occupational exposure.

References

Hazardous Substances Data Bank (HSDB, 1997). National Library of Medicine. Bethesda, MD.

Maronpot RR, Shimkin MB, Witschi HP, Smith LH, Cline JM (1986). Strain A mouse pulmonary tumor test results for chemicals previously tested in the National Cancer Institute carcinogenicity tests. *J Natl Cancer Inst* 76(6):1101-1112.

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C, Caspary WJ (1988). Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals.

NCI (1979). *Bioassay of trimethyllthiourea for possible carcinogenicity*. Technical report series no. 129. National Cancer Institute, US Department of Health, Education, and Welfare, National Institutes of Health, DHEW publication no. (NIH) 79-1384.

CARCINOGENICITY DATA SUMMARY: TRIS(2-ETHYLHEXYL)PHOSPHATE (TRIOCTYL PHOSPHATE)

Tris(2-ethylhexyl)phosphate (TEHP, trioctyl phosphate; CAS No. 78-48-2) is used as a fire retardant in consumer products, such as clothing, and as a plasticizer for products made using polyvinylchloride (PVC) resins. It is also used as a solvent. Production in the U.S. was estimated to be 3 million pounds in 1974 (HSDB, 1995).

Carcinogenicity Data available:

Epidemiological studies

No epidemiological studies of cancer rates in humans exposed to TEHP were found in the literature.

Animal bioassays

1. Rat 103-week gavage studies: NTP, 1984; Kluwe *et al.*, 1985; Kluwe, 1986. TEHP dissolved in corn oil was administered by intragastric intubation to groups of 50 male and 50 female F344/N rats 5 days per week for 103 weeks. Male rats were given daily doses of: 0, 2,000 or 4,000 mg per kg body weight; female rats were given: 0, 1,000 or 2,000 mg/kg body weight. In male rats, the incidence of benign and malignant (combined) pheochromocytomas of the adrenal gland was 2/50, 9/50 and 14/50 in control, low- and high-dose groups, respectively. The incidences in both the low- and high-dose males were significantly increased above the incidence in controls ($p=0.026$ and $p=0.001$, respectively), but they were not significantly above the incidence of pheochromocytomas in historical control male F344/N rats used in the NTP program. NTP concluded that there was equivocal evidence of carcinogenicity in male F344/N rats given TEHP. No significant increases in tumor incidence were found in female rats given TEHP.

2. Mouse 103-week gavage studies: NTP, 1984; Kluwe *et al.*, 1985; Kluwe, 1986. Groups of 50 male and female $B6C3F_1$ mice received TEHP in corn oil by intragastric intubation at doses of 0, 500 or 1,000 mg/kg body weight 5 days per week for 103 weeks. In females, the incidence of hepatocellular carcinomas was 0/48, 4/50 and 7/50 in control, low- and high-dose animals, respectively. The incidence in the high-dose was significantly increased above the incidence in controls ($p=0.007$). NTP concluded that there was some evidence of carcinogenicity in female $B6C3F_1$ mice given TEHP. No significant increases in tumor incidence were found in male mice given TEHP.

Other relevant data

TEHP increased the frequency of sister-chromatid exchange (SCE) in a nonstandard protocol experiment, but was negative in tests for chromosome aberrations, recessive lethal mutations in *Drosophila*, mutations in mouse lymphoma cells, micronuclei, and reverse mutations in *Salmonella typhimurium* (NTP, 1984).

TEHP has some structural similarity to the organophosphates, trimethylphosphate, tris(2-chloroethyl)phosphate and tris(2,3-dibromopropyl)phosphate, and also to di(2-ethylhexyl)phthalate, all of which are listed under Proposition 65 as substances known to the State to cause cancer.

Preliminary evaluation of carcinogenicity and exposure data:

Although TEHP did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern. This is associated with the increase in the incidence of hepatocellular carcinomas in female $B6C3F_1$ mice exposed to TEHP. There was also an increased incidence of benign and malignant pheochromocytomas of the adrenal gland in male F344/N rats. This increase was statistically significant when compared to concurrent controls, but not when compared to historical control male F344/N rats. The level of concern is supported by structural similarities with several known carcinogens.

There is a **HIGH** level of **concern over the extent of exposure** to TEHP because it is used as a fire retardant and plasticizer in consumer products.

References

Hazardous Substances Data Bank (HSDB, 1995). National Library of Medicine. Bethesda, MD.

Kluwe WM (1986). Carcinogenic potential of phthalic acid esters and related compounds. *Environ Health Perspect* **65**:271-278.

Kluwe WM, Huff JE, Matthews HB, Irwin R, Haseman JK (1985). Comparative chronic toxicities and carcinogenesis potentials of 2-ethylhexyl-containing compounds in rats and mice. *Carcinogenesis* **6**:1577-1583.

National Toxicology Program (NTP, 1984). Toxicology and Carcinogenesis Studies of Tris(2-ethyllhexyl)phosphate (CAS No. 78-42-2) in F344/N rats and B6C3F1 mice (Gavage studies). NTP Technical Report No. 274. NIH Publication No. 84-2530. National Toxicology Program, Research Triangle Park, NC.

CARCINOGENICITY DATA SUMMARY: 2-AMINO-5-NITROTHIAZOLE

2-Amino-5-nitrothiazole (ANT, 5-nitro-2-thiazolamine, aminonitrothiazolum, 5-nitro-2-aminothiazole; CAS No. 121-66-4) has been used since 1950 as a veterinary antiprotozoal agent for farm fowl and pigeons. It is used in the production of other antiprotozoal agents, and may be used as an intermediate in the preparation of disperse azo dyes (HSDB, 1994). Prior to 1980, it was used in animal feed (IARC, 1983). There is only one U.S. commercial producer, which reported 1977 production in the range of 4.5 - 45.4 thousand kg (9,921-100,089 pounds). IARC (1983) reviewed 2-amino-5-nitrothiazole and concluded that there were no adequate data available on the carcinogenicity in humans and there was limited evidence in animals (Group 3). Since this evaluation additional genotoxicity information has been published: the evaluation was listed but not updated in IARC (1987).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to 2-amino-5-nitrothiazole were found in the literature.

Animal bioassays

1. Mouse 104-week feeding studies: NCI, 1978. Groups of 50 $B6C3F_1$ mice/sex/dose were fed diets containing 0, 50 or 100 mg 2-amino-5-nitrothiazole/kg for 104 weeks. No treatment related tumors were observed in either sex.

2. Rat 111-week feeding studies: NCI, 1978. Groups of 50 Fischer 344 rats/sex/dose were fed diets containing 0, 300, or 600 mg 2-amino-5-nitrothiazole/kg for 110 weeks, followed by one week on control diet prior to terminal sacrifice. In males, there was a significant dose-related trend in cancers of the hematopoietic system, including the combined incidence of malignant lymphoma, lymphocytic leukemia and undifferentiated leukemia (22%, 30%, 39%, for controls, low- and high-dose groups, respectively; p=0.044), granulocytic leukemia (4%, 8%, 18%, p=0.014), and lymphoma and leukemia (combined) (26%, 38%, 57%, p=0.001). High-dose males had a statistically significant increase in granulocytic leukemia (p=0.023) and lymphoma and leukemia (combined) (p=0.002) as compared with controls. Chromophobe adenomas of the pituitary gland were significantly increased in female rats at both the low- (p=0.048) and high-doses (p=0.021) (42%, 62%, 66%), but only marginally increased in males. Due to the variable historical control incidence of chromophobe adenomas observed in both sexes of this strain of rat, NCI concluded that these pituitary adenomas were not clearly treatment-related. A significant increase (p=0.023) in the incidence of endometrial stromal polyps was observed in low-dose females (2/50, 9/49, 3/50). However, due to the absence of a dose-dependent response, the NCI concluded that these endometrial stromal polyps could not be clearly associated with 2-amino-5-nitrothiazole. NCI concluded that there was clear evidence of the carcinogenicity of 2-amino-5-nitrothiazole in male rats.

3. Female rat 66-week feeding study (46 weeks + 20 weeks observation): Cohen *et al*. 1975. Thirty-five female weanling Sprague-Dawley rats were fed 2-amino-5-nitrothiazole in the diet at 0.114% for one week, 0.075% for 8 weeks, 0.1% for 37 weeks, and then control diet for 20 weeks, for a total study duration of 66 weeks. Thirty-nine female rats were fed control diet. A significant increase ($p<0.03$) in the incidence of mammary tumors (fibroadenomas and adenocarcinomas combined) was observed in treated females (8/35; 7 fibroadenomas and 1 adenocarcinoma) as compared to controls (2/39; both were fibroadenomas).

Other Relevant Data

2-Amino-5-nitrothiazole is mutagenic in *Salmonella* with or without metabolic activation and in *E. coli* (Voogd *et al*., 1983). It was also mutagenic in the mouse lymphoma assay (Myhr and Caspary, 1988; Mitchell *et al*., 1988). 2-Amino-5-nitrothiazole is structurally related to carcinogenic nitrofurans, such as the 4-substituted 2-hydrazinothioazoles, which have been show to induce primarily mammary gland tumors in rats (Cohen *et al*., 1975).

Preliminary evaluation of carcinogenicity and exposure data:

Although did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern. This is associated with the induction of malignant cancers of the hematopoietic system in male F344 rats, and of primarily benign mammary tumors in female Sprague-Dawley rats. 2-amino-5-nitrothiazole also showed mutagenicity in bacterial and mammalian systems, and has structural analogies with rat tumorigens.

There is a **MEDIUM** level of **concern over the extent of exposure** to 2-amino-5-nitrothiazole since occupational exposure may occur as a result of the compound's manufacture, use as a veterinary drug, and use as a chemical intermediate in the synthesis of other antiprotozoal agents and disperse azo dyes.

References

Cohen SM, Ertürk E, Von Esch AM, Crovetti AJ, Bryan GT (1975). Carcinogenicity of 5-nitrofurans and related compounds with amino-heterocyclic substituents. *J Natl Cancer Inst* **54**:841-850.

Hazardous Substances Data Bank (HSDB, 1994). National Library of Medicine. Bethesda, MD.

International Agency for Research on Cancer (IARC, 1983). *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: Some food additives, feed additives and naturally occurring substances.* Volume 31, pp. 71-77. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of carcinogenic risks to humans. Overall evaluation of carcinogenicity: An updating of IARC monographs Volumes 1 to 42.* Supplement 7: p. 57. IARC, Lyon, France.

Mitchell AD, Rudd CJ, Caspary WJ (1988). Evaluation of the L5178Y Mouse Lymphoma Cell Mutagenesis Assay: Intralaboratory Results for Sixty-Three Coded Chemicals Tested at SRI International. *Environ Mol Mut* **12**(Suppl 13): 37-101.

Myhr BC, Caspary WJ (1988). Evaluation of the L5178Y Mouse Lymphoma Cell Mutagenesis Assay: Intralaboratory Results for Sixty-Three Coded Chemicals Tested at Litton Bionetics, Inc. *Environ Mol Mut* **12**(Suppl 13):103-194.

National Cancer Institute (NCI, 1978). Bioassay of 2-amino-5-nitrothiazole for Possible Carcinogenicity. CAS No. 121-66-4. Technical Report Series No. 53. US Department of Health and Human Services, Bethesda, MD.

Voogd CE, van der Stel JJ, Verhaven HW (1983). The capacity of some nitro- and amino-heterocyclic sulfur compounds to induce base-pair substitutions. *Mutat Res* **118**:153-165.

CARCINOGENICITY DATA SUMMARY: 11-AMINOUNDECANOIC ACID

11-Aminoundecanoic acid (CAS No. 2432-99-7) is an initiator for type 11 nylons which are used in the automotive industry, in industrial fabrics (filter bags, work clothes, netting), and brushes due to its resistance to vibration and shock and its stability when in contact with fuels. Nylon-11 accounts for <1% of nylon use in the US. The FDA has stated that nylon-11 may be safely used to produce articles intended for use in processing, handling, and packaging food (21CFR177.1500). IARC (1985) reported that there was only one major industrial producer of 11-aminoundecanoic acid and this producer is based in France and exports to a U.S. subsidiary for polymer production. 11-Aminoundecanoic acid is reportedly not produced in the US (NTP, 1982), but is listed on US EPA's 1993 TSCA Chemical Inventory. A NIOSH (1983) survey estimated that 1,534 workers may be exposed to this compound.

IARC has categorized 11-aminoundecanoic acid in Group 3 (unclassifiable) as to its carcinogenicity to humans based upon no data in humans and limited data from experimental animals (IARC, 1985; IARC, 1987). Additional genotoxicity information has become available since the IARC (1985) review.

Carcinogenicity Data available:

Epidemiological studies

No data regarding the carcinogenicity of 11-aminoundecanoic acid in humans were located in the scientific literature.

Animal bioassays

1. Mouse long-term feeding studies: NTP, 1982. B6C3F1 mice (50/sex/dose) were fed diet containing 0, 7500 or 15,000 ppm 11-aminoundecanoic acid in their diet for 103 weeks. A dose-related decrease in body weight and survival was observed among mice of both sexes, with mortality >50% in male mice. Among low-dose male mice, a significant increase in malignant lymphomas was observed (2/50 control; 9/50 low-dose; 4/50 high-dose; $p<0.05$) (Life table analysis of the high-dose group was not significant). The NTP concluded that "no clear evidence was found for the carcinogenicity of 11-aminoundecanoic acid in B6C3F1 mice of either sex, although the increase in malignant lymphoma in male mice may have been associated with administration of 11-aminoundecanoic acid."

2. Rat long-term feeding studies: NTP, 1982. F344 rats (50/sex/dose) were fed diet containing 0, 7500, or 15,000 ppm 11-aminoundecanoic acid in their diet for 104 weeks. A significant decrease in weight gain and survival was observed among male rats. Male rates showed a significant increase in neoplastic nodules in the liver in both treated groups (1/50 control; 9/50 low-dose; 8/50 high-dose; $p<0.01$). Transitional cell carcinomas of the urinary bladder (0/48 control; 0/48 low dose; 7/49 high dose; $p<0.01$) and hyperplasia of the transitional cell epithelium of the urinary bladder were also significantly increased in male rats. An increased incidence (2%, 2%, 10%) of calculi of the urinary bladder was observed in male rats that had no urinary bladder tumors. Transitional cell carcinomas of the urinary bladder were not observed in any of 780 historical controls. The NTP concluded that 11-aminoundecanoic acid was carcinogenic to male rats.

Other relevant data

11-Aminoundecanoic acid did not induce sex-linked recessive mutation in *Drosophila melanogaster* or UDS in hepatocytes of rats treated *in vivo* (Yoon et al., 1985; Mirsalis et al., 1983). It did, however, induce transformation in BALB/C-3T3 cells in newly developed co-culture clonal survival assay (Matthews, 1993). In a summary of the genotoxicity of 11-aminoundecanoic acid, NTP reported it to be negative for chromosome aberrations (unreferenced), positive for SCE (citing Galloway et al., 1987), negative in the mouse lymphoma assay (citing McGregor et al., 1988), negative in the micronucleus assay (unreferenced), and negative in *Salmonella* assays (citing Mortelmans et al., 1986).

Preliminary evaluation of carcinogenicity and exposure data

Although 11-aminoundecanoic acid did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern associated with the evidence for liver and bladder tumor

development in male rats. Transitional cell carcinomas of the urinary bladder are extremely rare in control rats. It is unclear what role the formation of bladder calculi may have in the development of tumors in the case of this compound. Male mice also showed an increase in the incidence of malignant lymphomas, although this effect did not show a clear dose relationship; however, the early mortality in the high-dose group likely precluded the observation of a dose-dependent tumor increase. The mixed genotoxicity results neither add to nor reduce the level of concern.

There is a **MEDIUM** level of **exposure concern** regarding 11-aminoundecanoic acid because of the potential for occupational exposure. There is little concern regarding exposure of the general public to this compound. Food contact products manufactured using nylon-11 are unlikely to contain significant amounts of the 11-aminoundecanoic acid in the final product.

References

Galloway SM, Armstrong MA, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin BH, Resnick M, Anderson B, Zeiger E (1987). *Environ Mol Mutagen* **10**(Suppl 10):1-176.

International Agency for Research on Cancer (IARC, 1985). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Chemicals Used in Plastics and Elastomers*. Vol. 39, pp. 239-45. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42.* Supplement 7. IARC, Lyon, France.

Matthews EJ (1993). Transformation of BALB/c-3T3 cells: III. Development of a co-culture clonal survival assay for quantification of chemical cytotoxicity in high-density cell cultures. *Environ Health Perspect* **101**(Suppl 2): 311-8.

McGregor D, Brown A, Cattanach P, Edwards I, McBride D, Riach C, Caspary W (1988). Responses of the L5178 tk+/tk- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environ Mol Mutagen* **12**: 85-154.

Mirsalis J, Tyson K, Beck J, Loh E, Steinmetz K, Contreras C, Austere L, Martin S, Spalding J (1983). Induction of unscheduled DNA synthesis (UDS) in hepatocytes following *in vitro* and *in vivo* treatment [Abstract No. Ef-5]. *Environ Mutagen* **5**: 482.

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ Mutagen* **8**(Suppl 7):1-119.

National Institute for Occupational Safety and Health (NIOSH, 1983). National Occupational Hazard Survey. NIOSH, Cincinnati, OH.

National Toxicology Program (NTP, 1982). Carcinogenesis bioassay of 11-aminoundecanoic acid (CAS No. 2432-99-7) in F344 rats and B6C3F1 mice (feed study). TR-216. Research Triangle Park, NC.

Yoon JS, Mason JM, Valencia R, Woodruff RC, Simmering S (1985). Chemical mutagenesis testing in *Drosophila*. IV. Results of 45 coded compounds tested for the National Toxicology Program. *Environ Mutagen* **7**:349-67.

CARCINOGENICITY DATA SUMMARY: ANTIPYRINE (PHENAZONE)

Antipyrine (phenazone; CAS No. 60-80-0) is a prescription pyrazolone analgesic (with local anesthetic action) that is currently used in combination with other drugs to alleviate pain/inflammation in the middle ear (acute otitis media), primarily in children. Application of antipyrine is topical (PDR, 1997). Antipyrine has been associated with 4 industries and 163 facilities, with an estimated 1,907 employees (1,486 women) occupationally exposed (RTECS, 1997, citing NIOSH, 1983). At one time, antipyrine was used as an oral analgesic.

Carcinogenicity Data available:

Epidemiological studies

In an international case-control study on the relationship between analgesics and renal cell-cancer, the authors concluded that there was no evidence for a positive association with pyrazolones (including antipyrine) (McCredie et al., 1995). The relative risk for women (3 cases, 2 controls) consuming >5.0 kg over a lifetime before 1987 was 2.8 (95% CI 0.4 - 18.1), with adjustments for study center, age, gender, body-mass index, and pack-years of tobacco. Cases were subjects aged 20-79 diagnosed with renal-cell cancer between 1989 and 1991; control subjects were frequency matched by age and gender.

Animal bioassays

1. Rat long-term feeding study: Johansson, 1981. Groups of 30 male Sprague-Dawley rats received diet containing 0 or 0.535% antipyrine for up to 117 weeks. Among treated rats, invasive renal pelvic tumors occurred in 4/29 rats compared to 0/30 controls (p=0.052). The tumors consisted of 2 urothelial tumors invading the kidney and 2 squamous-cell carcinomas that were invading the kidney and hilar fat tissue. The treated group also had an incidence of 5/29 tumors or papillomatosis of the urinary bladder compared to 2/30 in the control group (p=0.20). Two of the bladder tumors in each group were associated with severe urinary tract infection. This study also included groups receiving antipyrine in combination with phenacetin and caffeine, and with phenacetin alone. Tumors of the renal pelvis also developed in these groups.

2. Rat long-term feeding study: Johansson and Anderström, 1988. Groups of 30 male Sprague-Dawley rats received diet containing antipyrine (0.535%), 0.2% N-[4-(5-nitro-2-furyl)-2thiozolyl] formamide (FANFT) for 5 weeks followed by 0.535% antipyrine, 0.535% antipyrine with 0.102% caffeine, or no added chemicals for 88 weeks. The administration of antipyrine alone or in combination with caffeine did not result in any urinary tract tumors. In animals first administered FANFT then antipyrine, but not FANFT alone, tumors of the renal pelvis (5/27) and urinary bladder (5/27) were significantly increased over control animals (p=0.02).

3. Rat long-term feeding promotion study: Shibata et al., 1995. Male rats were initiated with 0.1% dihydroxy-di-N-propyl nitrosamine in water and 3% uracil in the diet for 4 weeks. One week after cessation of this treatment, 1% antipyrine was given in the diet for 35 weeks. Another group of rats received only antipyrine for 35 weeks with 5 weeks additional observation. Antipyrine administration without initiation did not induce tumors, but following initiation enhanced the incidence of renal cell tumors, hyperplasic lesions of the renal pelvis and ureter, pre- or neoplastic lesions of the urinary bladder, induction of hyperplastic lesions of the ureter, and pre- and neoplastic lesions of the liver.

Other relevant data

Dietary antipyrine (0.5, 1, or 1.5%) has been shown to lead to cell proliferation of the urothelium and degenerative changes in urothelial cells in rats (Johansson et al., 1989).

Antipyrine tested negative in the histidine reversion-Ames test (RTECS, 1996; citing EPA Genetox Program, 1988). Antipyrine was found to inhibit DNA synthesis in HeLa cells (DI_{50} = 80 mM; Heil and Reifferscheid, 1992).

Preliminary evaluation of carcinogenicity and exposure data

Although antipyrine did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern associated with the marginal evidence for the development of kidney tumors in male rats. Antipyrine also appears to have some potential in tumor promotion, particularly in the liver and the lower urinary tract. There is no evidence of genotoxicity to support this level of concern.

There is a **MEDIUM** level of **exposure concern** regarding antipyrine because of its use as a pharmaceutical in the treatment of pain caused by middle ear infections; however, the frequency of use for this indication is thought to be relatively low. Little systemic exposure would be likely following topical application. There is also some concern from potential occupational exposure.

References

Heil J, Reifferscheid G (1992). Detection of mammalian carcinogens with an immunological DNA synthesis-inhibition test. *Carcinogenesis* **13**(12):2389-2394.

Johansson SL (1981). Carcinogenicity of analgesics: Long-term treatment of Sprague-Dawley rats with phenacetin, phenazone, caffeine and paracetamol (acetamidophen). *Int J Cancer* **27**(4): 521-529.

Johansson SL, Anderström C (1988). The influence of antipyrene on *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide-induced urinary tract carcinogenesis. *Carcinogenesis* **9**(5): 783-787.

Johansson SL, Radio SJ, Saidi J, Sakata T (1989). The effects of acetaminophen, antipyrine and phenacetin on rat urothelial cell proliferation. *Carcinogenesis* **10**(1):105-111.

McCredie M, Pommer W, McLaughlin JK, Stewart JH, Lindblad P, Mandel JS, Mellemgaard A, Schlehofer B, Niwa S (1995). International renal-cell cancer study. II. Analgesics. *Int J Cancer* **60**:345-9.

National Institute for Occupational Safety and Health (NIOSH, 1983) National Occupational Hazard Survey (NOHS). Cincinnati, OH.

Physicians' Desk Reference (PDR, 1997). 51st Edition. Montvale, NJ: Medical Economics Company. p. 2810, 2476.

Registry of Toxic Effects of Chemical Substances (RTECS, 1997). Database produced by the U.S. National Institute for Occupational Safety and Health. Version date 1/97.

Shibata MA, Sano M, Hagiwara A, Hasegawa R, Shirai T (1995). Modification by analgesics of lesion development in the urinary tract and various other organic of rats preferred with dihydroxy-di-N-propylnitrosamine and uracil. *Japan J Cancer Res* **86**(2):160-167.

CARCINOGENICITY DATA SUMMARY: TROYSAN POLYPHASE (IPBC)

Troysan polyphase (IPBC, 3-iodo-2-propynyl ester butyl-carbamic acid; CAS No. 55406-53-6) is a pesticide used as a wood preservative and sealer. NIOSH estimated that in 1983 approximately 95,645 employees covering 56 industries and 5996 facilities were potentially exposed (RTECS, 1997). This agent is subject to TSCA Section 8(6) chemical inventory requirements. This chemical is registered for use as a pesticide in California.

Carcinogenicity Data available:

Epidemiological studies

No human carcinogenicity studies were found in the literature.

Animal bioassays

1. Mouse 78-week feeding studies: Inveresk Research, Scotland 1989, as summarized by US EPA, 1993. Technical grade troysan polyphase was fed to male and female CD-1 mice at doses of 0, 20, 50 and 150 mg/kg-d for 78 weeks. The numbers of animals were not reported in the summary information. Decreased body weight gain of 10% to 20% was observed for both sexes in the two highest dose groups for weeks 0 to 13 in the study. A statistically significant increase in hepatocellular adenoma was observed in high-dose males. A non-significant increase in pulmonary carcinomas was observed in high-dose females (10%) relative to controls (4%). Non-neoplastic changes to the thyroid were also noted, including atrophic follicular vacuolation, follicular coalescence and general follicular enlargement in all treated groups of both sexes.

2. Rat 104-week feeding studies: Inveresk Research, Scotland 1989, as summarized by US EPA, 1993. Technical grade troysan polyphase was fed to male and female Sprague-Dawley rats at doses of 0, 20, 50 and 80 mg/kg-d for 104 weeks. Decreased body weight gain (10 to 20%) was noted for the rats in the high-dose groups relative to controls for weeks 0 to 13 of the study. No treatment related tumors were observed in either sex.

Other relevant data

Troysan polyphase was negative in *Salmonella* mutagenicity tests for strains TA1535, TA1537, TA1538, TA98 and TA100 (US EPA, 1993) and in *E. coli* (US EPA, 1993). Troysan polyphase induced increases in aberrant colonies (DNA damage/repair), but no gene conversion or reverse mutations in yeast. It was negative in a mutagenic-DNA repair test in *B. subtilis*, negative in a mutagenic-unscheduled DNA synthesis assay in rat hepatocytes, and negative in a micronucleus assay in mice (US EPA, 1993).

Preliminary evaluation of carcinogenicity and exposure data:

Troysan polyphase has not been placed on the candidate list. In male mice fed this compound, benign liver tumors were observed in the high-dose group only. Tumorigenicity findings were negative in rats and female mice, and there were generally negative findings for genotoxicity.

There is a **MEDIUM** level of **concern over the extent of exposure** because troysan polyphase is a registered pesticide in California and is used in occupational settings.

References

Registry of Toxic Effects of Chemical Substances (RTECS, 1997). Database produced by the U.S. National Institute for Occupational Safety and Health. Version date 1/97.

U.S. Environmental Protection Agency (US EPA, 1993). Office of Prevention, Pesticides and Toxic Substances. TOX ONELINERS, Troysan KK-108A, P.C. Code 107801, 3-iodo-2-propynyl butylcarbamate, September 7, 1993.

CARCINOGENICITY DATA SUMMARY: *p*-BENZOQUINONE DIOXIME

p-Benzoquinone dioxime (*p*-quinone dioxime; CAS No. 105-11-3) has been reported to be used as a vulcanizing agent, chemical intermediate and bactericide. Its primary use has been reported to be in the production of butyl rubber. It is also used in the production of EPDM elastomers (IARC, 1987). Current estimates of U.S. production of this chemical were not available; however, HSDB (1994) provided estimates for 1972 and 1975 of approximately 450 kg/year. This agent was approved for use by FDA in 1980 for rubber articles in contact with food (IARC, 1987). *p*-Benzoquinone dioxime was classified by IARC (1982, 1987) as a Group 3 carcinogen, based on limited evidence of carcinogenicity in animals.

Carcinogenicity Data available:

Epidemiological studies

No human carcinogenicity studies were reported by IARC (1982, 1987) or found in a search of the more recent scientific literature by OEHHA.

Animal bioassays

1. Rat 104-week feeding studies: NCI, 1979. Fischer 344 rats (50 animals/sex/group) were fed *p*-benzoquinone dioxime at concentrations of 375 and 750 ppm. Twenty animals of each sex served as controls. Dosing was continued for 104 weeks. In female rats, tumors of the urinary bladder (transitional-cell papillomas) were observed with incidences of 0/19, 1/43 and 4/44 for the control, low- and high-dose groups, respectively. Urinary bladder transitional-cell carcinoma was also increased in female rats, with incidences of 0/19, 1/43 and 7/44, respectively. These increases were statistically significant by the Cochran-Armitage trend test but not by the pairwise Fisher exact test. In male rats, tumors of the urinary bladder (transitional-cell papillomas) were elevated in the high-dose group (0/20, 0/45 and 2/46), but this increase was not statistically significant. The IARC Working Group (1982) noted that the NCI report made no mention of whether NCI researchers looked for the presence of bladder or renal calculi.

2. Mouse 104-week feeding studies: NCI, 1979. $B6C3F_1$ mice (50 animals/sex/group) were fed *p*-quinone dioxime at concentrations of 750 and 1500 ppm. Twenty animals of each sex served as controls. Dosing was continued for 104 weeks. A significantly increased trend in the incidence of hepatocellular carcinoma was observed in female mice. Incidences were 0/20, 3/41, and 5/50 for the control, low- and high-dose females, respectively (with an additional three liver neoplastic nodules in the high-dose group). NCI discounted these findings, noting that Fisher exact tests were not significant and that the historical control incidence of liver tumors in female mice was 4% and control incidences of 17% had been observed on occasion. No treatment-related tumors were observed in male mice.

Other relevant data

p-Benzoquinone dioxime was mutagenic in *S. typhimurium* (multiple studies) and mutagenic in the mouse lymphocyte forward mutation assay (RTECS, 1994; CCRIS, 1994). Negative results were reported for mutagenicity in *E. coli* (CCRIS, 1994). *p*-Benzoquinone dioxime was negative in an *in vivo* rat bone marrow micronucleus test and in a rodent liver unscheduled DNA synthesis assay (CCRIS, 1994).

Preliminary evaluation of carcinogenicity and exposure data:

Although *p*-benzoquinone dioxime did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern. This is associated with the positive evidence of urinary bladder tumor induction in female rats, and equivocal evidence of liver tumor induction in female mice. There are negative findings in male rats and mice. The level of concern also reflects the positive genotoxicity findings in a number of test systems.

There is a **LOW** level of **concern over the extent of exposure** because of the apparently low level of production and use of *p*-benzoquinone dioxime in the U.S.

References

Chemical Carcinogenesis Research Information System (CCRIS, 1994). Record 388.

Hazardous Substances Data Bank (HSDB, 1994). National Library of Medicine. Bethesda, MD.

International Agency for Research on Cancer (IARC, 1982). *Para*-benzoquinone dioxime. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Some Industrial Chemicals and Dyestuffs*. Volume 29, pp. 185-191. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity: an updating of IARC monographs volumes 1 to 42*. Suppl. 7. IARC, Lyon, France.

National Cancer Institute (NCI, 1979). Bioassay of *p*-quinone dioxime for possible carcinogenicity. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health. DHEW Publication No. (NIH) 79-1735.

Registry of Toxic Effects of Chemical Substances (RTECS, 1997). Database produced by the U.S. National Institute for Occupational Safety and Health. Version date 1/97

CARCINOGENICITY DATA SUMMARY: 4-CHLORO-4'-AMINODIPHENYL ETHER

4-Chloro-4'-aminodiphenyl ether (CADPE; 4-chlorophenoxy-aniline; 4-(4-chlorophenoxy)benzenamine; CAS No. 101-79-1) is an aromatic amine which has been identified as a pesticide (Weisburger *et al.* 1978), although it was not found in the 1995 California Pesticide Use Report (CDPR, 1995). It is, however, listed on the 1993 US EPA TSCA Chemical Inventory.

Carcinogenicity Data available:

Epidemiological studies

No data regarding the carcinogenicity of CADPE to humans have been located in the scientific literature. Some epidemiological studies have been conducted to investigate cancer incidence among individuals exposed to chlorophenoxy herbicides, but none focuses on exposure to CADPE.

Animal bioassays

1. Mouse long-term feeding studies: Weisburger *et al.*, 1978. Albino CD-1 mice (25/sex/dose) were fed diet containing 4000 or 8000 mg/kg CADPE hydrochloride for 3 months, followed by a decrease in feed concentrations to 2000 or 4000 mg/kg CADPE hydrochloride for 15 months. Among female mice, bladder tumors developed in 4/18 high-dose animals and 0/17 low-dose animals. This tumor type was not observed in 15 simultaneous or 102 pooled control animals ($p=0.075$ for the high-dose group, relative to simultaneous control group; $p<0.01$, relative to pooled control group). The tumors were described as "transitional cell and squamous papillomas and one unusual tumor which combined malignant epithelial and sarcomatous elements". Vascular tumors were observed in 3/18 high-dose and 5/17 low-dose female mice compared to 2/15 simultaneous and 9/102 pooled control mice ($p=0.03$ for the low-dose group relative to pooled controls). No tumors were observed in male mice. This study had small groups (n=25) and the compound was administered for less than 2 years (18 months). No consistent dose-response was observed. No information on survival was provided.

2. Rat long-term feeding study: Weisburger *et al.*, 1978. Male Charles River rats (25/dose) were fed CADPE hydrochloride in their diet at doses of 2000 and 4000 mg/kg diet for 3 months followed by 15 months at 1000 and 2000 mg/kg diet for 15 months. A statistically significant ($p<0.025$) increase in hepatocellular carcinomas was observed in the low-dose group (4/13 vs. 0/16 simultaneous control animals or 2/111 pooled control animals), but not the high-dose group (1/23). "Multiple tumors" were also increased significantly in the low-dose group (5/13 vs. 2/16 simultaneous controls or 14/111 pooled control animals), but not the high-dose group (2/23). These tumors included tumors of the pituitary, stomach, liver, kidney, and bladder.

Other relevant data

CADPE has been reported to cause mutations in *Salmonella typhimurium* (Miyauchi *et al.*, 1983). CADPE belongs to the class of aromatic amines, some of which have been shown to be carcinogenic to the bladder and at other sites in humans and experimental animals.

Preliminary evaluation of carcinogenicity and exposure data

Although CADPE did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern. The reporting of primarily benign bladder tumors in female mice treated with CADPE is cause for some concern because of the rarity of this tumor type. This increase had statistical significance only relative to a pooled control group. Liver and vascular tumors were increased in the low-dose groups of rats and mice, respectively, although there was no clear evidence of a dose-response relationship. No data were presented which might account for this observation (such as early mortality in the high-dose group). There is some supporting evidence for the mutagenicity of CADPE, as well as a structural similarity to compounds (aromatic amines) known to be carcinogenic.

There is a **LOW** level of **concern regarding exposure** to CADPE. There is no indication that this chemical is used as a pesticide, or for any other purpose, in California. It is not known whether any residues are currently present in imported foods.

References

California Department of Pesticide Regulation (CDPR, 1995). 1995 Pesticide Use Report.

Miyauchi M, Haga M, Takou Y, Uematsu T (1983). Mutagenic activity of chlorinated 4-nitrobiphenyl ethers and their nitroso- and amino-derivatives. *Chem Biol Interact* **44**(1-2): 133-141.

Weisburger EK, Russfield AB, Homburger F, Weisburger JH, Boger E, Van Dongen CG, Chu KC (1978). Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J Environ Path Tox* **2**:325-356.

CARCINOGENICITY DATA SUMMARY: 4-CHLORO-*m*-PHENYLENEDIAMINE

4-Chloro-*m*-phenylenediamine (CAS No. 5131-60-2) is an aromatic amine which is used an intermediate in dye production, as an agent in rubber processing, and in chemical research (IARC, 1982; HSDB, 1994). It has been patented for hair dye use. U.S. import estimates from 1970 were approximately 4500 pounds (IARC, 1982). The European Communities allow meta-phenylenediamines in cosmetics at concentrations of up to 6% in the finished products. No data were available regarding US production or exports (HSDB, 1994).

IARC (1982) determined that the available data were inadequate to evaluate the carcinogenicity of 4-chloro-*m*-phenylenediamine in experimental animals (Group 3; IARC, 1987). NCI (1978) concluded that dietary administration of 4-chloro-*m*-phenylenediamine was carcinogenic to animals, but these studies were evaluated by IARC and found to provide inadequate evidence for carcinogenicity in animals. This was primarily because of discrepancies between experimental and historical control incidences (IARC, 1987). The positive genotoxicity studies reported by Dunkel *et al.* (1985) and Watanabe (1989) were not evaluated by IARC; a more recent evaluation using current guidelines would consider these studies.

Carcinogenicity Data available:

IARC (1982) determined that the results of the studies with 4-chloro-*m*-phenylenediamine in mice were inconclusive and those in rats were not indicative of a carcinogenic effect. The IARC review did not include the original data from the studies reported by Milman and Peterson (1984), Dunkel *et al.* (1985), or Maronpot *et al.* (1986). These original study data, in addition to the data reviewed by IARC, are briefly described below.

Epidemiological studies

No studies of the long-term effects of human exposure to 4-chloro-*m*-phenlyenediamine have been reported, although the NCI (1978) stated that they selected this compound for study because of increased incidences in cancer in dye workers who are exposed to this compound (among others).

Animal bioassays

1. Rat long-term feeding studies: NCI, 1978; Weisburger *et al.*, 1980. Groups of 49-50 male and 50 female F344 rats were fed dietary concentrations of 0, 0.2, or 0.4% 4-chloro-*m*-phenylenediamine for 78 weeks. Among male rats receiving 0.4 percent 4-chloro-*m*-phenylenediamine, a significant ($p = 0.011$) increase in the incidence of adrenal pheochromocytomas was observed. The incidences of adrenal pheochromocytomas were 4/46, 7/48, and 14/48 in the control, low-dose, and high-dose groups, respectively. Zymbal's gland tumors occurred in 2 male and 1 female high-dose animals. IARC (1982) noted that the observed differences do not appear to be significant when historical controls are taken into account. The NCI concluded that dietary administration of 4-chloro-*m*-phenylenediamine was carcinogenic to male Fischer 344 rats.

2. Mouse long-term feeding studies: NCI, 1978; Weisburger *et al.*, 1980. Groups of 49-50 male and 50 female mice were fed time-weighted average dietary concentrations of 0, 0.7, or 1.4% 4-chloro-*m*-phenylenediamine for 78 weeks. Among female mice receiving 0.7% 4-chloro-*m*-phenylenediamine, a significant ($p = 0.002$) increase in the incidence of hepatocellular carcinomas was observed. The incidences of hepatocellular carcinomas were 0/46, 8/44, and 5/45 in the control, low-dose, and high-dose groups, respectively. Significantly increased combined incidences of hepatocellular carcinomas and hepatocellular adenomas in both experimental groups were also observed. The combined carcinoma and adenoma incidences were 0/46, 11/44 ($p < 0.001$), and 8/45 ($p = 0.003$) in the control, low-dose, and high-dose groups, respectively. IARC (1982) noted that the incidence of liver tumors in the control group was lower than that observed in historical controls and that the incidence in the treated groups was within the normal range seen in historical controls. The NCI concluded that dietary administration of 4-chloro-*m*-phenylenediamine was carcinogenic to female B6C3F1 mice.

3. Mouse intraperitoneal screening studies: Maronpot *et al.*, 1986. Strain A mice (10/sex/group) were injected with 104, 198, or 283 mg of 4-chloro-*m*-phenylenediamine/kg body weight in tricaprylin 3 times a week for 8 weeks. No tumors were observed in male mice of any dose group. Incidences of lung tumors among female

mice were 11%, 0%, 11%, and 25% in vehicle control, low-dose, mid-dose, and high-dose groups, respectively. Based on these incidences, the authors concluded that 4-chloro-*m*-phenylenediamine was non-genotoxic when injected in strain A mice.

Other relevant data

Milman and Peterson (1984) reviewed the Weisburger *et al.* (1978) study and a number of NCI studies on phenylenediamines and related compounds and concluded that, in general, ring-substituted 1,3-phenylenediamines (including 4-chloro-*m*-phenyleneidamine) and related compounds are carcinogenic. In this review article, eight of the 12 ring-substituted 1,3-phenylenediamines and related compounds that were examined were found to be carcinogenic. The results of a computerized analysis of structure-activity relationships based on a set of rules generated by US EPA experts (Oncologic®, version 2.40) finds that 4-chloro-*m*-phenylenediamine is of high-to-moderate concern. This is the highest level of concern noted for chemicals not included in the database of carcinogenicity bioassay results from which the program rules are derived.

4-Chloro-*m*-phenylenediamine caused mutations in *Salmonella typhimurium* with and without metabolic activation by S9 (Dunkel *et al.*, 1985). Watanabe *et al.* (1989) found that, when treated with hydrogen peroxide, m-phenylenediamine and one of its derivatives, 2,4-diaminoanisole, were mutagenic in *Salmonella typhimurium* but only with metabolic activation by S9.

Preliminary evaluation of carcinogenicity and exposure data

4-Chloro-*m*-phenylenediamine did not reach a level of carcinogenic concern sufficient to be placed on the candidate list. However, there is some carcinogenicity concern, associated with the increases (relative to concurrent controls, but within the historical range) in incidences of adrenal pheochromocytomas in male rats and of hepatocellular tumors in female mice. This concern is also based on the positive genotoxicity studies, and the structural relationships to known carcinogens, including a computerized analysis which predicts that 4-chloro-*m*-phenylenediamine is of high-to-moderate concern.

There is a **LOW** level of **concern regarding exposure** to 4-chloro-*m*-phenylenediamine. There was no information suggesting a current use for this compound in the US. However, if European formulation standards were to apply in the US (up to 6% in finished products such as hair dyes), exposure potential would be much higher.

References

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, Rosenkranz HS, Simmon VF (1985). Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ Mutagen* **7** (Suppl 5):1-248.

Hazardous Substances Data Bank (HSDB, 1994). National Library of Medicine. Bethesda, MD.

International Agency for Research on Cancer (IARC, 1982). *IARC monographs on the evaluation of carcinogenic risk of chemicals to humans: Some aromatic amines, anthraquinones and nitroso compounds, and inorganic fluorides used in drinking-water and dental preparations.* Volume 27. IARC, Lyon, France

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of carcinogenic risks to humans, Overall evaluation of carcinogenicity: An updating of IARC monographs Volumes 1 to 42.* Supplement 7. IARC, Lyon.

Maronpot RR, Shimkin MB, Witschi HP, Smith LH, Cline JM (1986). Strain A mouse pulmonary tumor test results for chemicals previously tested in the National Cancer Institute carcinogenicity tests. *J Natl Cancer Inst* **76**:1101-1112.

Milman HA, Peterson C (1984). Apparent correlation between structure and carcinogenicity of phenylenediamines and related compounds. *Environ Health Perspect* **56**: 261-273.

NCI (1978). National Cancer Institute. Bioassay of 4-chloro-*m*-phenylenediamine for possible carcinogenicity. Technical Report Series No. 85. DHEW Publication No. (NIH) 78-1335. NCI, Bethesda, MD.

Oncologic®: An expert system that evaluates the carcinogenic potential of chemicals. LogiChem Inc., P.O. Box 357, Boyertown, PA 19512.

Watanabe T, Hirayama T, Fukui S (1989). Phenazine derivatives as the mutagenic reaction product from o- or m-phenylenediamine derivatives with hydrogen peroxide. *Mutat Res* **227**:135-145.

Weisburger E, Russfield AB, Homburger F, Weisburger JH, Boger E, Van Dongen CG, Chu KC (1978). Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J Environ Path Toxicol* **2**:325-56.

Weisburger EK, Murthy AS, Fleischman RW, Hagopian M (1980). Carcinogenicity of 4-chloro-o-phenyleneidamine, 4-chloro-*m*-phenylenediamine, and 2-chloro-p-phenylenediamine in Fischer 344 rats and B6C3F1 mice. *Carcinogenesis* **1**(6): 495-9.

CARCINOGENICITY DATA SUMMARY: DIBROMOMANNITOL

Dibromomannitol (1,6-dibromo-1,6-dideoxy-D-mannitol; Mitobronitol; Myelobromol; Myebrol; CAS No. 488-41-5) is a cancer chemotherapeutic agent used primarily in the management of thrombocythaemia, both primary, and secondary to chronic myeloid leukemia or polycythemia vera (Reynolds, 1989). The recommended daily administered dose ranges from 0.25 to 1 g dibromomannitol by mouth for adults. Dibromomannitol is not listed in the 1997 Physician's Desk Reference (PDR, 1997), but is listed in the Martindale Pharmacopoeia (Reynolds, 1989) and the Merck Index (Budavari *et al.*, 1996).

Carcinogenicity Data available:

Epidemiological studies

No data regarding the carcinogenicity of dibromomannitol in humans were located in the scientific literature.

Animal bioassays

1. Mouse long-term injection studies (Weisburger *et al.*, 1975): Swiss Webster mice (25/sex/dose) were injected intraperitoneally with 90 or 180 mg/kg/dose dibromomannitol 3 times/week for 6 months followed by a 12 month holding/observation period. Groups of untreated (101 male, 153 female) and vehicle (numbers not stated) controls were included. It was noted that survival was poor among high-dose male mice. Reported tumor incidences combined the findings from both dose groups. Among treated mice, lung tumors were reported in 8/25 males. Untreated male control mice were reported to develop alveolar cell adenomas (4/101) and adenocarcinomas (6/101). The authors report a p-value of 0.006 for lung tumors in male mice. (This would correspond to an incidence of 9/101 in the control group, a plausible number for combined adenomas and adenocarcinomas). Among treated female mice, both lung tumors (19/40) and lymphomas (9/40) were reported. Control female mice developed alveolar cell adenomas (7/153) and adenocarcinomas (14/153) and disseminated lymphosarcoma (1/153). Reported p-values for the treatment groups were both <0.001. The authors considered the effect on the male lung "borderline".

2. Rat long-term injection studies (Weisburger *et al.*, 1975): CD rats (25/sex/dose) were injected intraperitoneally with 125 or 250 mg/kg/dose 3 times/week for 6 months followed by a 12 month holding/observation period. A pool of untreated animals (179 males, 181 females) served as controls. The authors noted poor survival among both male and female treated rats. Reported tumor incidences combined the findings from both dose groups. Peritoneal sarcomas (6/37 treated vs. 0/179 control; $p<0.001$) and subcutaneous tumors (6/37 treated vs. 0/179 control; $p<0.001$) were significantly increased in male rats. In female rats, peritoneal sarcomas were significantly increased (2/40 vs. 0/181 control; $p=0.032$).

Other relevant data

The principal mechanism of action of dibromomannitol as a chemotherapeutic agent is alkylation via an epoxide intermediate (Reynolds, 1989). As an alkylating agent, this agent has the potential to damage DNA.

In an *in vivo* assay in mice, dibromomannitol administered intravenously induced a moderate increase in sister chromatid exchanges (SCE) and chromosomal aberrations (Nakanishi and Schneider, 1979). Dibromomannitol was shown to be mutagenic in *Salmonella typhimurium* strains TA1535 and TA100 and to induce SCE in Chinese hamster ovary cells (Oláh *et al.*, 1983). Dibromomannitol was found to induce sex-linked recessive lethal mutations and reciprocal translocations in *Drosophila melanogaster* (Foureman *et al.*, 1994). Dibromomannitol has been shown to inhibit DNA synthesis in rabbit bone marrow and inhibit the incorporation of ^{14}C-uridine into RNA of human tonsillar cells *in vitro* (Hidvegi *et al.*, 1976). An NTP summary of dibromomannitol genetic toxicity indicated that the compound tested positive *in vitro* for chromosomal aberrations and SCE, in the micronucleus assay, and in *Salmonella* (NTP, 1998; citing Mortelmans *et al.*, 1986).

Preliminary evaluation of carcinogenicity and exposure data

Although dibromomannitol did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern associated with the evidence for tumor development in both rats and mice. Dibromomannitol-treated mice developed tumors at sites distal from the site of administration (intraperitoneal). The possibility of effects dependent upon the route of exposure and limited reporting of data limit the conclusions which can be drawn from the primary study. The action of dibromomannitol has been shown to be similar to that of the direct alkylating agents, in that it induced cancer both at the site of injection (i.e., peritoneal cavity and subcutaneous tissue) and at distant sites. This level of concern includes consistent findings of genotoxicity.

There is a **LOW** level of **exposure concern** regarding dibromomannitol. This compound has potential use as a chemotherapeutic agent, but it does not appear to be used in the US (PDR, 1997).

References

Budavari S, O'Neil MJ, Smith A, Heckelman PE, Kinneary JF, eds (1996). Mitobronitol. *The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals*. 12th ed. Whitehouse Station, NJ: Merck & Co. p. 1062.

Foureman P, Mason JM, Valencia R, Zimmering S (1994). Chemical mutagenesis testing in *Drosophila*. X. Results of 70 coded chemicals tested for the National Toxicology Program. *Environ Mol Mutagen* **23**(3):208-27.

Hidvegi EJ, Sebestyen J, Szabo LD, Koteles GJ, Institoris L (1976). The effect of dibromo-dulcitol, diepoxy dulcitol and various new cytostatic hexitol derivatives on the metabolic activities of nucleic acids and proteins--II. *Biochem Pharmacol* **25**(15):1705-1710.

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E (1986). *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ Mutagen* **8**(Suppl 7):1-119.

Nakanishi Y, Schneider EL (1979). In vivo sister-chromatid exchange: a sensitive measure of DNA damage. *Mutat Res* **60**(3):329-337.

National Toxicology Program (NTP, 1998). Dibromomannitol. Last Updated 5/8/98. Available at: http:\\ntp-server.niehs.nih.gov/htdocs/Results_status/ResstatD/10837-D.html.

Oláh E, Tóth K, Sugár J, Hegedüs L, Somfai-Relle S (1983). Effects of some sugar alcohol derivatives on mutation and induction of sister chromatid exchanges. *Cancer Res* **43**(10):4530-4536.

Physician's Desk Reference (PDR, 1997). Medical Economics Company, Inc.: Montvale, NJ.

Reynolds JEF, ed (1989). Mitobronitol. *Martindale - The Extra Pharmacopoeia*. London: The Pharmaceutical Press. p. 641.

Weisburger, JH, Griswold DP, Prejean JD, Casey AE, Wood HB, Weisburger EK (1975). The carcinogenic properties of some of the principal drugs used in clinical cancer chemotherapy. *Recent Results in Cancer Research* **52**:1-17.

CARCINOGENICITY DATA SUMMARY: MEXACARBATE

Mexacarbate (Zectran; 4-dimethylamino-3,5-xylylmethylcarbamate; CAS No. 315-18-4) is registered for use in the United States as an insecticide and a molluscicide against lawn, turf, and flower pests. It is not registered for use on food crops (Handbook of Fate and Exposure Data, 1991). However, mexacarbate has been discontinued by Dow Chemical Company as a registered pesticide (since ~1975) and has not been identified in any other uses (Farm Chemicals Handbook, 1992). Less than 10 pounds of this chemical were used as a pesticide in 1995 in the state of California for landscape maintenance and treatment of other greenhouse and ornamental plants (Pesticide Use Report, 1995; CDPR, 1992). CDPR does not list any active products containing mexacarbate (no products containing mexacarbate have been registered in California since 1989). No data are available concerning exposures to the general population or concentrations in environmental media (Handbook of Fate and Exposure Data, 1991). Human exposure to mexacarbate would be expected to result primarily from its use as a pesticide for the control of lawn, turf, and flower pests, most likely via inhalation or dermal contact.

IARC concluded that "the available evidence do not permit an evaluation of the carcinogenicity of zectran to experimental animals" (Group 3; IARC, 1976; IARC, 1987), although the more recent studies (NCI, 1978) were not available in their evaluation.

Carcinogenicity Data available:

Epidemiological studies

No data concerning carcinogenic effects of mexacarbate in humans were located in the literature.

Animal bioassays

1. Mouse long-term feed studies: NCI, 1978. B6C3F_1 mice (50/sex/group, plus 20/sex as controls) were fed diet containing 0, 327, or 654 ppm (male) and 0, 68, or 135 ppm (female) mexacarbate (time-weighted average concentration) for 78 weeks followed by 14-15 weeks of observation. Poor survival among the male control mice required statistical analysis of tumor incidence to be performed with a pooled control group. The incidences of hepatocellular carcinoma, subcutaneous fibrosarcoma, and skin fibromas showed a significant trend with dietary concentrations among male mice surviving at least 56 weeks, although the incidences of tumors were not significantly elevated over controls. NCI stated that the maximum tolerated dose of mexacarbate may not have been reached with the female mice. NCI concluded there was insufficient evidence for the carcinogenicity of mexacarbate in B6C3F_1 mice.

2. Rat long-term feed studies: NCI, 1978. Osborne-Mendel rats (50/sex/group, plus 20/sex as controls) were fed diet containing 0, 209, or 418 ppm (male) and 0, 339, or 678 ppm (female) mexacarbate (time-weighted average concentration) for 78 weeks followed by 14-15 weeks of observation. No treatment-related tumors were observed among the rats. NCI concluded that there was not sufficient evidence of carcinogenicity for Osborne-Mendel rats.

3. Mouse long-term gavage/feed studies: Innes et al., 1969; as reviewed in IARC, 1976. (C57BL/6×C3H/Anf)F_1 and (C57BL/6×AKR)F_1 mice (18/sex/group) were treated with commercial grade mexacarbate (95%) by stomach tube at 4.64 mg/kg body weight from 1 to 4 weeks of age (dose unadjusted after first treatment), and subsequently in their diet at 11 mg/kg to age 78 weeks. Total tumor and hepatoma incidences were increased among male mice of the first strain ($p<0.05$). Lung adenoma incidences were increased among male and female mice of the first strain (males - 6% vs. 25%; females - 3% vs. 18%; $p<0.01$ for both sexes combined). Hepatoma incidence was also increased among males in the second strain ($p<0.05$).

4. Mouse long-term injection studies: Innes et al., 1969; as reviewed in IARC, 1976. Month-old (C57BL/6×C3H/Anf)F_1 and (C57BL/6×AKR)F_1 mice (18/sex/group) were treated with commercial grade mexacarbate (95%) once subcutaneously and then observed for 78 weeks. No treatment-related tumors were observed.

Other relevant data

Reverse mutations were increased in *Bacillus subtilis* without metabolic activation upon exposure to mexacarbate (IARC, 1976; DeGiovanni-Donnelly *et al.*, 1968). Human liver preparations metabolized mexacarbate (67%) mainly by oxidation of substituents attached to the phenyl ring. This compound was asssigned a final level of carcinogenicity concern of LOW by the Oncologic program based on test data and SAR considerations.

Preliminary evaluation of carcinogenicity and exposure data

Although mexacarbate did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern associated with the evidence showing the development of treatment-related hepatomas in two mouse strains. Lung adenomas were also increased among male and female mice of one strain. A third study in mice (NCI, 1978) showed a dose-related trend in incidence of hepatocellular carcinoma in mice, although the individual incidences were not significantly increased relative to the controls. Additional studies in rats and mice were negative. There is some evidence for the genotoxicity of mexacarbate in bacteria.

There a **LOW** level of **concern over the extent of exposure** to mexacarbate. While applications of this pesticide continue to be reported, the quantity used is extremely low. This chemical is expected to be phased out completely as private stocks are depleted. Dow Chemical has discontinued this pesticide and does not currently sell it. Present use levels are negligible when considering general exposure.

References

DeGiovanni-Donnely R, Kolbye SM, Greeves PD (1968). The effects of IPC, CIPC, sevin and zectran on *Bacillus subtilis*. *Experientia* **24**:80-81.

Farm Chemicals Handbook (1992): Mexacarbate. Meister Publishing Company. Willoughby, OH.

Handbook of Fate and Exposure Data for Organic Chemicals (1991). Mexacarbate. Volume III. Howard, P.H. ed. Lewis Publishers. Chelsea, MI.

Innes JRM, Ulland BM, Valerio MG, Petrucelli L, Fishbein L, Hart ER, Pallotta AJ, Bates RR, Falk HL, Gart JJ, Klein M, Mitchell I, Peters J (1969). Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. *J Natl Cancer Inst* **42**:1101-1114.

International Agency for Research on Cancer (IARC, 1976). Zectran. *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Some carbamates, thiocarbamates and carbazides.* Vol 12, pp. 237-243. IARC, Lyon, France

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of carcinogenic risks to humans, Overall evaluation of carcinogenicity: An updating of IARC monographs Volumes 1 to 42.* Supplement 7, p.74. IARC, Lyon.

National Cancer Institute (NCI, 1978). Bioassay of mexacarbate for possible carcinogenicity. NCI-TR-147. Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, NCI, National Institutes of Health, Bethesda, MD. DHEW Publ. No. (NIH) 78-1703.

Pesticide Use Report (1995). State of California Environmental Protection Agency, Department of Pesticide Regulation, Information Systems Branch. Sacramento, CA.

CARCINOGENICITY DATA SUMMARY: TRIBENURON-METHYL

Tribenuron-methyl (Express®, Grandstar®, DPX-L 5300®; CAS No. 101200-48-0) is an herbicide. The chemical can be used to control broadleaf weeds. It is not registered for use in California (CDPR, 1998).

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Mouse 18-month feeding studies: Haskell Laboratory 1987, referenced in US EPA, 1997. Mice were exposed to 0, 20, 200, and 1500 ppm of tribenuron-methyl in the diet for 18 months. No increase in tumor incidence was reported.

2. Rat 2-year feeding studies: Haskell Laboratory 1987, referenced in US EPA, 1997. Groups of 72 male and female rats were exposed to 0, 25, 250, and 1250 ppm of tribenuron-methyl in the diet for 2 years. There were statistically significant trends in mammary gland adenocarcinomas and in adenomas and/or adenocarcinomas. The incidence rates of mammary gland adenocarcinomas and adenomas and/or adenocarcinomas of female rats exposed to 1250 ppm were also significantly higher than those of the controls.

Other Relevant Data

Tribenuron-methyl was negative in the Ames test either with or without metabolic activation. It did not induce structural or numerical chromosome damage in primary human lymphocytes exposed to levels up to a toxic level (100 µg/ml). The chemical was also negative in the micronuclei assay and in an unscheduled DNA synthesis assay (US EPA, 1997).

Preliminary evaluation of carcinogenicity and exposure data:

Although tribenuron-methyl did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern associated with the elevated incidence of malignant mammary gland tumors in female rats. Based on the negative results of a number of short-term tests, the chemical does not appear to be genotoxic.

There is a **LOW** level of **concern over the extent of exposure** as the chemical is not currently registered for use as a pesticide in California (CDPR, 1998).

References

California Department of Pesticide Regulation (CDPR, 1998). California Environmental Protection Agency. Homepage at http://www.cdpr.ca.gov. May, 1998.

U.S. Environmental Protection Agency (US EPA, 1997). Tox-Oneliner for Express®. Office of Pesticide Programs, October, 1997.

CARCINOGENICITY DATA SUMMARY: 6-METHOXY-2-NITRONAPHTHO[1,8-bc]PYRAN

6-Methoxy-2-nitronaphtho[1,8-bc]pyran (CAS No. 10502-39-9) is a chemical used in research laboratories.

Carcinogenicity Data available:

Epidemiological studies

No data on carcinogenic effects of human exposure to this chemical were identified.

Animal bioassays

1. Rat 22-week subcutaneous injection study: Salmon *et al.*, 1989. Fifteen male Wistar rats were administered 6-methoxy-2-nitronaphtho[1,8-bc]pyran via subcutaneous injection once a week for 22 weeks. The total amount of chemical injected was approximately 10.2 mg per test animal. There were 10 male rats in the control group: 5 animals were injected with the solvent alone and another 5 animals received no injection. Every animal injected with 6-methoxy-2-nitronaphtho[1,8-bc]pyran developed soft tissue sarcomas at the site of injection. The average time for tumor appearance was 116 days. No tumors were found at the site of injection among the controls.

Other Relevant Data

6-Methoxy-2-nitronaphtho[1,8-bc]pyran was mutagenic to *Salmonella typhimurium* TA100 without metabolic activation, but not to *Salmonella typhimurium* TA98, with or without metabolic activation (Salmon *et al.*, 1989). The chemical induced DNA amplification in hamster cells *in vitro* (Royer *et al.*, 1986). 6-Methoxy-2-nitronaphtho[1,8-bc]pyran is chemically related to the nitro-naphthofuran compounds; many of these compounds are direct mutagens and carcinogens to laboratory animals.

Preliminary evaluation of carcinogenicity and exposure data:

Although 6-methoxy-2-nitronaphtho[1,8-bc]pyran did not reach a level of carcinogenic concern sufficient to be placed on the candidate list, there is some carcinogenicity concern associated with the induction of tumors at the site of subcutaneous injection. This level of concern is also based on the mutagenic potential of the chemical in bacteria and mammalian cells *in vitro*, and the structural relationship to nitro-naphthofurans, a group of chemicals known for their mutagenicity and carcinogenicity in laboratory animals.

There is **NO IDENTIFIED CONCERN over the extent of exposure** to 6-methoxy-2-nitronaphtho[1,8-bc]pyran, since it is used solely as a laboratory research chemical.

References

Royer R, Buisson JP, Vleminckx C, Moens W (1986). Sur de nouveaux réactifs puissamment génotoxiques: les dérivés nitrés de l'oxaphénalène. *Eur J Med Chem - Chim Ther* **21**:351-354.

Salmon RJ, Buisson JP, Hendrickx B, Vielh Ph, Aussepe L, Moens W, Royer R (1989). *In-vivo* carcinogenicity of 2-nitro-oxaphenalenes. *Carcinogenesis* **10**(5):803-805.

CARCINOGENICITY DATA SUMMARY: 1-BUTANOL

1-Butanol (n-butanol, n-butyl alcohol; CAS No. 71-36-3) is used as a solvent for oils, dyes, alkaloids, fats, waxes, resins, shellac, varnishes, and rubbers; as a chemical intermediate in the manufacture of pharmaceuticals, hydraulic fluids, detergent formulations, ethylene glycol monobutyl ether, plasticizers, butylamines, 2,4-D esters, and butyl acrylate; as a bactericide in veterinary use; as a fabric coating; as a flavoring agent in non-alcoholic and alcoholic beverages, ice cream, ices, candy, cream, and baked goods; as a medication for major throat problems (HSDB, 1995), and as a pesticide formulation ingredient on a wide variety of food crops, including various grains, fruits, and vegetables (CDPR, 1996). 1-Butanol occurs naturally as a product of the fermentation of carbohydrates (IPCS, 1987).

There are six U.S. manufacturers of 1-butanol. Production in the U.S. in 1993 was 1.33 billion pounds (HSDB, 1995). The National Institute for Occupational Health and Safety (NIOSH) estimated in the National Occupational Exposure Survey of 1983 that 852,067 employees in 49,987 facilities were potentially exposed to 1-butanol (RTECS, 1995). In 1994, 24,353 pounds were reported used for agricultural purposes in California, while in 1995, agricultural use was reported at 7,383 pounds (CDPR, 1995; 1996). California Toxics Release Inventory data indicate that 141,922 pounds were released in 1994.

The US EPA classified 1-butanol as a group D carcinogen (not classifiable as to human carcinogenicity, based on the absence of human or animal cancer data) in 1991 (US EPA, 1995).

Carcinogenicity Data available:

Epidemiological studies

No data on long-term effects of human exposure to 1-butanol were found in the literature.

Animal bioassays

No data from animal cancer bioassays of 1-butanol were found in the literature.

Other Relevant Data

1-Butanol weakly inhibited DNA synthesis in *E. coli* (US EPA, 1995) and induced spindle disturbances and aneuploidy in hamster V79 lung cells (Önfelt, 1987). It was negative in *Salmonella* mutation assays, sister chromatid exchange assays in chick embryos and Chinese hamster ovary cells, and an *in vitro* micronucleus assay in Chinese hamster V79 cells (US EPA, 1995). An unpublished *in vivo* NMRI mouse bone marrow micronucleus assay conducted on behalf of the Chemical Manufacturers Association (provided to OEHHA in June 1998) was also negative.

1-Butanol is known to increase the fluidity of cellular membranes (Salyer *et al*., 1990), and Önfelt (1987) suggested that this physico/chemical property may account for the induction of spindle disturbances and aneuploidy seen in V79 cells *in vitro*. Therefore, the spindle effects and aneuploidy seen in hamster V79 lung cells may not be indicative of a potential for 1-butanol to be genotoxic *in vivo*.

Preliminary evaluation of carcinogenicity and exposure data:

There are **INADEQUATE DATA** to assign a level of **carcinogenicity concern** for 1-butanol since no animal or human carcinogenicity studies are available. Data from genotoxicity assays are mainly negative.

There is a **HIGH** level of **concern over the extent of exposure** to 1-butanol since it is produced in very large quantities, released into the California environment in large quantities, and widely used. In addition to occupationally exposed populations, the general population is exposed as a result of 1-butanol's use as a flavoring agent and pesticide formulation ingredient, and its natural occurrence in foods and beverages.

References

California Department of Pesticide Regulation (CDPR, 1995). Annual Pesticide Use Report, 1994. Cal/EPA/DPR. Sacramento, CA.

California Department of Pesticide Regulation (CDPR, 1996). Annual Pesticide Use Report, 1995. Cal/EPA/DPR. Sacramento, CA.

Hazardous Substance Data Bank (HSDB, 1995). National Library of Medicine. Bethesda, MD.

International Programme on Chemical Safety (IPCS, 1987). Butanols –Four isomers: 1-butanol, 2-butanol, tert-butanol, isobutanol. *Environ Health Criteria* **65**:9-42.

Önfelt A (1987). Spindle disturbances in mammalian cells. III. Toxicity, c-mitosis and aneuploidy with 22 different compounds. Specific and unspecific mechanisms. *Mutat Res* **182**:135-154.

Registry of Toxic Effects of Chemical Substances (RTECS, 1995). Database produced by the U.S. National Institute for Occupational Safety and Health. Version date 7/95: RTECS number E01400000.

Salyer JL, Bohnsack JF, Knape WA, Shigeoka AO, Ashwood ER, Hill HR (1990). Mechanisms of tumor necrosis factor-alpha alteration of PMN adhesion and migration. *Am J Pathol* **136**:831-41.

United States Environmental Protection Agency (USEPA, 1995). Integrated Risk Information System (IRIS®). Environmental Criteria and Assessment Office, USEPA, Cincinnati, OH.

CARCINOGEN DATA SUMMARY: 2-BROMO-2-METHYLPROPANE

2-Bromo-2-methylpropane (*tert*-butyl bromide; trimethylbromomethane; 2-bromoisobutane; CAS No. 507-19-7) is a low molecular weight alkyl halide used mainly as an alkylating agent in chemical research and synthesis. It may also be among the brominated trihalomethane compounds formed from reactions occurring during chlorine disinfection and in bromide contaminated drinking water (Batjer *et al.*, 1980). In the U.S., s*ec*-butyl bromide, a similar compound, was found in drinking water experimentally treated with chlorine (Thompson *et al.*, 1990).

Carcinogenicity Data available:

Epidemiological studies

No studies on the long-term effects of human exposure to 2-bromo-2-methylpropane have been reported.

Animal bioassays

1. Mouse 24-week intraperitoneal (i.p.) injection studies: Poirier *et al.*, 1975. A/Heston strain male and female mice were injected i.p. 3x/week for 8 weeks with total doses of 8.7, 21.8 and 43.7 mmoles 2-bromo-2-methylpropane/kg, and sacrificed at 24 weeks. There was a slight but significant increase ($p<0.05$) in pulmonary adenomas in the two highest dose groups. Lung tumor incidences were 6/29, 8/15, 8/15, and 6/9 in untreated controls, low-, mid-, and high-dose groups, respectively. The authors concluded that 2-bromo-2-methylpropane may be weakly carcinogenic.

Other relevant data

2-Bromo-2-methylpropane was not mutagenic in *E. coli* without S-9 activation (Leifer *et al.*, 1981). 2-Bromo-2-methylpropane was mutagenic when tested in the Ames assay with desiccators to control volatility (Simmon *et al*., 1977).

Preliminary evaluation of carcinogenicity and exposure data:

There are **INADEQUATE DATA** to assign a level of **carcinogenicity concern** over 2-bromo-2-methylpropane. The lack of a clear dose-response, the poor survival of the dosed animals, the small number of animals per group, the incomplete reporting of tumor incidence by sex, and the moderate tumorigenic response in the 24-week Strain A mouse lung adenoma study conducted by Poirier *et al*. (1975), together with one positive and another non-positive report from *in vitro* bacterial mutagenicity assays, do not provide adequate information to make a preliminary evaluation of the carcinogenicity of 2-bromo-2-methylpropane.

There is a **MEDIUM** level of **concern over the extent of exposure** to 2-bromo-2-methylpropane. It may potentially be present in drinking water, formed as a result of chlorine disinfection (Batjer *et al.*, 1980). It is used in chemical synthesis (HSDB, 1996), and as a basic research chemical. It is predicted to be absorbed through the skin (HSDB, 1996).

References

Batjer K, Gabel B, Koschorrek M, Lahl U, Lierse KW, Stachel B, Thiemann W (1980). Drinking water in Bremen: trihalomethanes and social costs. A case study of bromoform formation during chlorination of river water highly contaminated with bromide ions. *Sci Total Environ* **14**(3):287-91.

Hazardous Substances Data Bank (HSDB, 1996). National Library of Medicine. Bethesda, MD.

Leifer Z, Kada T, Mandel M, Zeiger E, Stafford R, Rosenkranz HS (1981). An evaluation of tests using DNA repair-deficient bacteria for predicting genotoxicity and carcinogenicity. A report of the U.S. EPA's Gene-TOX Program. *Mutat Res* **87**:211-297.

Poirier LA, Stoner GD, Shimkin MB (1975). Bioassay of alkyl halides and nucleotide base analogs by pulmonary tumor response in strain A mice. *Cancer Res* **35**:1411-1415.

Simmon VF, Kauhanen K, Tardiff RG (1977). Mutagenic activity of chemicals identified in drinking water. In: *Developments in Toxicology and Environmental Science* Vol. 2: Progress in Genetic Toxicology. Scott D, Bridges BA, Sobels FH (eds.) Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands pp. 249-258.

Thompson GP, Christman RF, Johnson JD (1990). Chlorination of Aquatic Fulvic Acid and Natural Waters: Additional By-Products. In: *Water Chlorination: Chemistry, Environmental Impact and Health Effects*. Jolley RL et. al. (eds.) Lewis Publishers Inc. Chelsea, Michigan.